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The Biology of *Anasa tristis* DeGeer

With Particular Reference to

The Tachinid Parasite, *Trichopoda pennipes* Fabr.

Raimon L. Beard

Connecticut
Agricultural Experiment Station
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This is a revision, with additions, of a dissertation presented to the Faculty of the Graduate School of Yale University in candidacy for the degree of Doctor of Philosophy in 1939.

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INTRODUCTION

This study is an outgrowth of a project begun in 1934 by the Connecticut Agricultural Experiment Station at New Haven. Originally designed to find an effective insecticide for use against the common squash bug (*Anasa tristis* DeG.), the project was begun by Mr. D. C. Elliott, but undertaken in 1935 by the author, who has continued the work to the present date. During 1936 and the three succeeding years, the work was expanded to embrace the studies presented in the pages to follow. These studies are chiefly concerned with the effect of a common Tachinid parasite (*Trichopoda pennipes* Fabr.) on the squash bug, considering not only the morphological and physiological relationships between the two insects, but also the effect of the population of the parasite upon that of the host. Such other information necessary for a more complete account or for a better understanding of these relationships is included.

Most of the field observations were made at the Mount Carmel farm of the Connecticut Experiment Station, although squash fields in the towns of New Haven (Westville), Meriden, and Southington were used freely. The laboratories of the Experiment Station were at the disposal of the writer, as were the facilities of Osborn Zoölogical Laboratory of Yale University, where he was a graduate student during the course of the investigation.

The author wishes to make grateful acknowledgment to Dr. R. B. Friend, under whose direction this work was done, for his helpful suggestions and valuable criticisms. The coöperation of Mr. Neely Turner in the management of the squash fields at the Experiment Station farm is sincerely appreciated. The author also wishes to express his gratitude for valuable suggestions and assistance in gathering data to numerous other members of the staff of the Experiment Station and members of Osborn Zoölogical Laboratory of Yale University.

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I. THE COMMON SQUASH BUG

GENERAL ACCOUNT

THE common squash bug, *Anasa tristis* DeG., is a well-known native insect. Its widespread occurrence on squash and related cucurbits has made it a familiar object to the home gardener and truck farmer, who may know it as the "stink bug", although this opprobrious name more properly belongs to bugs of the family Pentatomidae rather than to those of the family Coreidae, of which *A. tristis* is a member.

Typical of the Heteroptera (Order Hemiptera), and readily available, this insect has been more extensively studied probably than any other member of the group. Most of its morphology is well known, and its life history and habits have been widely publicized. Much of the description of the latter, however, is sketchy and frequently but a reiteration of a few papers more extensive in scope.

The original description and illustration of the squash bug are found in the writings of De Geer (1773) under the name *Cimex tristis*. This species was described under the name of *Cimex moestus* by Gmelin (in Linné, *Systema Naturae*, 1788). Tigny (1802) also listed "La Punaise triste" under this name. The *Coreus rugator* of Fabricius (1803) and the *Oriterus destructor* of Hahn (1831) have fallen as synonyms, as has *Coreus ordinatus* of Say (1825). The species *rugator* was placed in the genus *Gonocerus* by Burmeister (1835) and in *Homoeocerus* by Herrich-Schaeffer (1853). Dallas (1851) listed the species as *Gonocerus tristis*. The genus *Anasa* was erected by Amyot and Serville in 1843, and Stål (1862) was the first, apparently, to place *tristis* in this genus. Other early observers to list the squash bug as *Anasa tristis* include Uhler (1876), Provancher (1886), and Van Duzee (1897), although Jaeger (1864), Verrill (1868), and Packard (1875) referred to it as *Coreus tristis*. Glover (1877) clarified this nomenclature by commenting on the synonomy of *Gonocerus tristis*, *Coreus tristis*, and *Anasa tristis*.

Geographical Distribution

The squash bug is confined to the western hemisphere, where it is found from South America northwards into Canada. Its presence in South America is based on the authority of Uhler (1876) who recorded it from Brazil, although Distant (1881) noted that he never found it there. Uhler (1878) recorded its presence in the West Indian Islands, Central America, and Mexico. Dallas (1851) listed *Gonocerus tristis* from Honduras. In the United States the present distribution of the common squash bug is throughout the whole extent of the country (Uhler, 1878; Britton, 1919). In Canada it is recorded from Quebec (Provancher, 1886) and Ontario (Van Duzee, 1917).

Developmental Stages

Egg

The egg of *A. tristis* measures about 1.48 mm. in length and 1.02 mm. in width. In end view it appears triangular in shape due to the flattened sides and base. The base is concave, with a raised central portion which serves as the point of attachment to any surface on which the egg is deposited. The convex surface is ovoid. The chorion appears smooth except under magnification, where a reticulated sculpturing composed of regular hexagons is observable. When first deposited, the egg is white to yellow in color, but gradually darkens with age, becoming dark bronze at the time of hatching. With proper illumination, the developing embryo can be distinguished beneath the chorion. The embryo is always oriented with its dorsum along the base of the egg and with the head directed toward the broader end, where the line of eclosion is clearly seen, particularly in the older eggs.

Nymphs

In its post-embryonic development—first noted, described and illustrated by Chittenden (1899)—the squash bug passes through five nymphal instars. Due to the long period of oviposition of the bug, it is possible, during August and at least part of September, to find all stages of the bug at one time. (Plate 1, B)[1] illustrates these different stages.

Upon hatching, the squash bug nymph is an attractive creature, being brightly colored, with the abdomen green and with the antennae, head, thorax and legs red. Within a few hours the parts of the body colored red become black. The antennae and legs are quite hairy, and the abdominal tubercles are conspicuous. The first instar nymph measures about 2.5 mm. in length.

When the first molt is completed, the nymph measures about 3 mm. and again possesses the red coloration on the head, antennae, and legs, which soon darkens to black. The thorax and abdomen, however, soon assume a dark greenish-gray color. As this stage approaches the second molt, the abdomen becomes swollen and turns to a lighter pruinose gray. There is no evidence of any wing pads, but the abdominal tubercles are more conspicuous than in the first instar. A widening of the penultimate segment of the antennae is characteristic of this stage.

The abdomen and thorax of the third instar nymph are of a uniform pruinose gray stippled with darker points. The anterior and lateral parts of the thorax, the head, and the appendages are also darker in color. The thoracic segmentation is distinct and slight traces of the wing pads can be detected. A nymph of this stage is about 4 mm. long.

The fourth and fifth instar nymphs are readily distinguished from the earlier instars by their size and the relative development of the wing pads. The coloration is about the same as that of the third stage. The fourth instar measures 6 to 7 mm. in length, while the fifth instar is considerably larger, 9 or 10 mm. in length. The wing pads are easily observed in the fourth stage, but are not nearly so well developed as in the final nymphal instar. In both of these stages a marked increase in the width of the thoracic and abdominal regions is evident, and the abdominal tubercles are even more conspicuous than in earlier instars.

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[1] Plates are found at the back of the bulletin.

Adult

The adult squash bug measures from 14 to 16 mm. in length, the male being slightly smaller than the female. In color, the wing membrane is black, and the rest of the body a mottled brown, varying in shade from light to dark among different individuals. The body shape is well seen in Plate 1, C.

Morphology

Much of the morphology of the adult *A. tristis* has been studied. The general external morphology has been described by Tower (1913), who also described, in greater detail (1914), the mouth parts of this insect. Breakey (1936) has made a comprehensive histological study of the digestive system of the bug, and Moody (1930) has given a good account of the repugnatorial glands of the nymphs as well as of the adult. Snodgrass (1933) has described in detail the external genitalia of the female.

Inasmuch as the reproductive systems of both sexes and the external genitalia of the male have not received adequate treatment, these are discussed below.

Female Reproductive System

Each ovary of *A. tristis* consists of seven ovarioles of the acrotrophic type joined anteriorly by a suspensory ligament. The very extensive tracheation of the ovary arises from the second abdominal spiracle (abdominal segment III). In hibernating bugs the egg tubes are slender and appear underdeveloped, but soon after the bugs appear in the field in late spring, active growth takes place, and the egg tubes rapidly become distended with developing ova. Usually four or five eggs of increasing size can be seen distal to the germarium in each ovariole. During the period of rapid egg production, the lateral oviducts present a beaded appearance due to the contained eggs, which are clearly visible through the thin walls of the oviduct. The common oviduct is short and expands widely to open into the large genital chamber. The dorsal portion of the genital chamber evaginates to enclose the dome-shaped bursa copulatrix which is heavily sclerotized. An opening on the side of this bursa admits a slender tube which becomes convoluted in its course to the spermatheca. The accessory glands are slender branched tubules which open laterally to the genital chamber and between the bases of the second valvifers of the ovipositor.

Male Reproductive System

The testis* of the squash bug is pear-shaped in its broad dimensions and flattened in the plane which lies parallel to the body wall. It is brilliantly pigmented, being colored orange in the last nymphal instar and early part of adult life, but later darkening to a bright crimson. Slight longitudinal depressions show each testis to be made up of seven lobes which lie side by side in a single plane. As with the ovary, the testis is supplied with numerous trachea arising from the second abdominal spiracle. The vasa deferentia are slender ducts which expand posteriorly to form large seminal vesicles. These unite with the median ejaculatory duct. Dorsally, at this point of union, lies a comparatively large lobulated structure which is shown histologically to be a compact mass of convoluted tubules representing the

* A description of the testis is incorporated in the work of Paulmier (1899).

accessory glands. The ejaculatory duct is slender and follows a rather tortuous course from the region of the seventh abdominal segment through the genital segments.

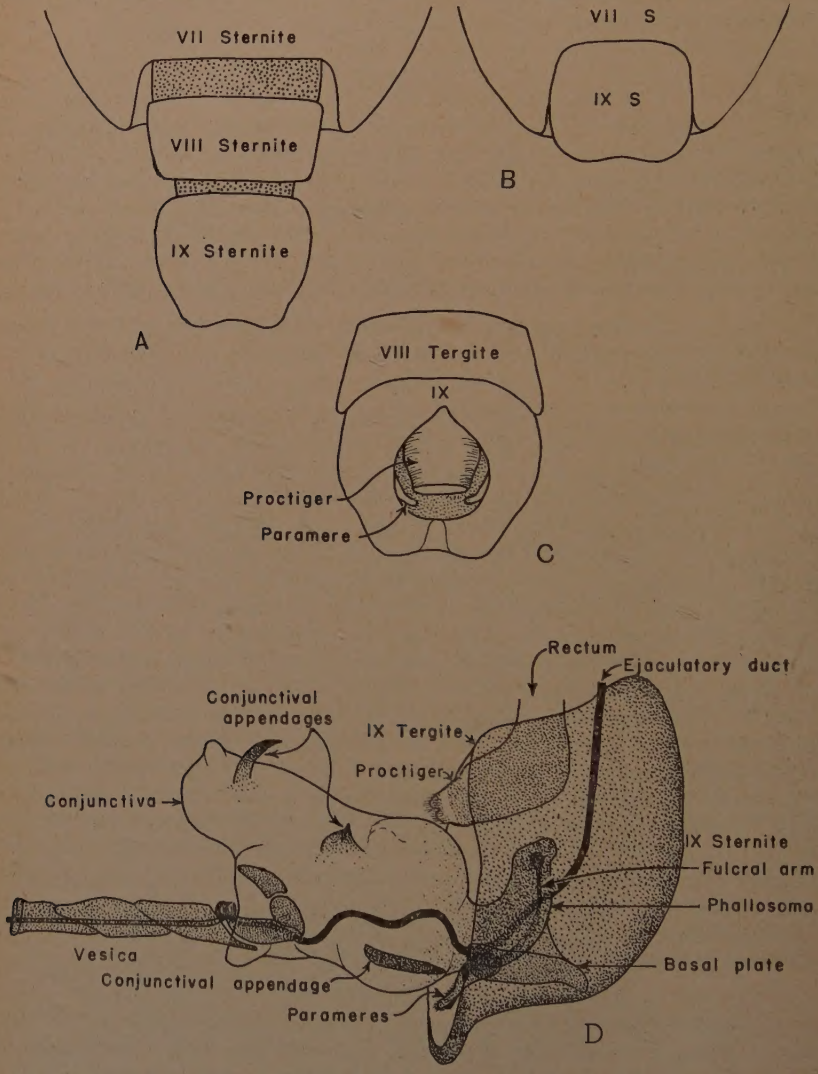

FIGURE 1. A. ♂ genital segments extended. B. ♂ genital segments in normal position, ventral aspect. C. Same, dorsal aspect. D. Fully extended ♂ genitalia in right lateral aspect.

Male Genitalia

Tower (1913) illustrated the external appearance of the genital segments, but numbered these segments erroneously. Snodgrass (1935) correctly numbered these, but gave no further description of the genitalia. Newell (1918)

figured certain portions of the genitalia, but her explanations are inadequate and her drawings are misleading. The genital organs of the male are complicated, but in general they compare favorably with those of other Heteroptera as described by Singh-Pruthi (1925), whose terminology is used in the following discussion.

The principal segment involved in the genitalia is the ninth. The eighth segment (Figure 1, A, B) is merely a collar which, at rest, is entirely telescoped into segment VII and which, in turn, encloses the anterior portion of segment IX. The sclerites of segment IX are completely fused into a heavily sclerotized, cup-shaped segment which encloses the retracted genitalia. The ninth tergite is less heavily sclerotized than the rest of the segment. On the dorsal aspect of the ninth segment (Figure 1, C) can be seen the protruding tubular proctiger, formed by the post-genital segments X and XI. Lateral to the proctiger protrude the tips of the parameres (called harpagones by Snodgrass, 1935), appendages of the ninth segment which serve a clasping function.

Figure 1, D shows diagrammatically the structural features of the fully extended genitalia in right lateral aspect.

The fused basal plates form a saddle-shaped sclerite, the convex surface of which conforms to the inner wall of the ninth sternite. Although its connection to the phallosoma is merely by a membranous sheet, it appears to move as a unit with the phallosoma. The dorsal arm of the latter has a pivotal articulation with the fulcral arm of the ninth sternite which originates at the base of the parameres. The latter, however, have an independent articulation with the ninth sternite. The phallosoma is strongly sclerotized and in anterior view is broadly triangular in shape, the ventral apex bearing an inner sclerotized ring through which the ejaculatory duct enters. Posteriorly, the phallosoma becomes less heavily sclerotized and expands into the membranous conjunctival portion of the endosoma. The conjunctiva is a large lobulated sac which bears three pairs of well-sclerotized appendages, which are not provided with muscles as are the parameres. The basal portion of the vesica is marked by a median, strap-shaped sclerite which is flanked on each side by a freely articulating lateral wing. When in a retracted state, these lateral wings swing around to occupy a more ventral position. The median portion bears ventrally a posteriorly directed arm. The ejaculatory duct enters the anterior end of this arm and emerges at the posterior end to coil twice before continuing in a straight line, terminating as the gonopore. The straight portion of the ejaculatory duct is surrounded by the peculiarly shaped vesica. This is strongly sclerotized and is in the form of a spiral band which completely encircles the duct three times.

When the genitalia are retracted, the vesica is drawn in straight, causing the conjunctiva to invaginate. Thus the conjunctival appendages are turned inside, and the anterior part of the vesica and most of the conjunctiva are enclosed by the sheath formed of the phallosoma and the basal plates. The whole aedeagus then rotates somewhat to occupy a position ventral to the proctiger.

Life History

In Connecticut *Anasa tristis* has one generation each year. The winter is passed by the hibernating adult bugs, and mating takes place in the spring. During the month of June the bugs seek squash fields, there to feed and oviposit. The oviposition period extends throughout the summer, beginning in early June and continuing until late August or early September.

The eggs of the squash bug are laid in masses, usually on the under surface of the leaves of the food plants. They commonly are spaced very uniformly and occupy angles formed by the leaf veins, but neither the spacing nor position are constant characteristics.

The duration of the egg stage of *A. tristis* varies considerably depending upon weather conditions. On isolated squash grown under natural conditions in 1938, observations indicated a maximum duration of 17 days for the first eggs deposited, and a minimum of 10 days for those deposited the last of July. The majority of the eggs hatched in 12 days. Under laboratory conditions maintained at a constant temperature of 74 degrees F. and a relative humidity of 62 percent, 11 days were required. These data may be compared with the insectary rearings by Elliott (1935), who observed that the time spent in the egg stage varies from 9 to 12 days with an average of 10 days.

The time required for the completion of the nymphal stages varies widely. This variation is associated not so much with weather conditions and the time of the season as with individual differences among the insects. Very few individuals pass through the five nymphal instars in less than about four weeks, and some individuals may require twice this length of time. Adding the duration of the egg stage, 36 days appear to be the minimum time in which the egg to adult life cycle can be completed in this locality.

The first nymphal instar uniformly requires three days for its completion, whether under natural or laboratory conditions. The second instar is more variable, usually requiring from five to eight days. The same length of time is spent in the third instar. Both the fourth and fifth stages require a somewhat longer time, but the variation is very wide and no satisfactory averages can be presented for natural conditions. The following summary of Elliott (1935) compares his data for insectary-reared material with those given by Worthley (1923) for other localities:

TIME (DAYS) REQUIRED FOR DEVELOPMENT OF *A. tristis*

	Connecticut		Massachusetts	New Hampshire	Wash. D. C.	Kansas
	Time	Mean				
Egg	9–12	10	13.7	11	9–10	7–17
1st instar	3	3	3.3	3	3	2.1–5.2
2nd instar	6–11	7	6.6	9	8–9	6.2–9.2
3rd instar	5–9	7	6.6	8	7–8	8.1–13
4th instar	6–16	8.3	6.4	7	6	10
5th instar	7–17	9	16.6	9	8	12
Total	36–67	44.3	53.2	47	41–44	45.4–66.4

Host Plants and Feeding Injury

Members of the family Cucurbitaceae, which includes such plants grown for their ornamental or edible fruits as squash, cucumber, pumpkin, melon, and gourds, are subject to attack by the squash bug. Normally the insect confines its feeding to this group, with a decided preference for squash, but in one case it has been reported (Rosewall, 1920) to be feeding in considerable numbers on the leaves and fruit of a fig tree in Louisiana. Girault (1906) mentioned squash bug nymphs feeding on grass.

Elliott (1935) planted a series of cucurbits to determine which plants are preferred in Connecticut. These included summer squash, Hubbard squash, pumpkin, cucumber, citron, watermelon, and muskmelon. Of these, summer squash, Hubbard squash, and pumpkin were the favored host plants. Adult bugs attacked these in the field and oviposited upon them. The other members of the group were not attacked at all, and squash bug nymphs placed upon cucumber, citron, and melon fed very little and died in the second or third instar.

Knowlton (1933) in Utah and Hoerner (1938) in Colorado noted that winter varieties of squash are most subject to attack by the bug. In Connecticut, the bug is commonly found on Hubbard squash, but in far fewer numbers than on summer squash. The reason for this may lie in the fact that summer squash is planted earlier than the winter variety and so serves as a trap crop in attracting the bugs as they emerge from hibernation.

Because of the commercial importance of these host plants, the squash bug is considered an economic pest. It has even been reported (Knowlton, 1933) that in some districts squash can scarcely be grown because of the ravages of this insect. Without doubt, however, the injury caused by this insect has been widely exaggerated. It is true that, when present in large numbers, the bug causes extensive damage, but doubtless much of the injury attributed to the squash bug is actually caused by the striped cucumber beetle (*Diabrotica vittata* Fabr.), the squash vine borer (*Melittia satyriniformis* Hbn.) and the wilt disease caused by the bacterium *Bacillus tracheiphilus*. It is probably seldom that the bug alone completely destroys a planting of squash, but it may be at times the contributing factor most directly responsible.

A misconception of the nature of the injury caused by the bug plus the conspicuousness of the insect have led to this exaggeration. Even reliable publications have illustrated bug injury by showing a general wilted condition of the entire food plant. While this is possible, if concerted feeding by a number of bugs occurs on the stem at the base of the plant, it is not the typical effect. Young seedlings bearing only cotyledon leaves or a very few small true leaves are easily killed and may show a wilted condition. In such cases the result is due to stem-feeding. Prolonged feeding even in one spot by a single bug causes a marked constriction in the stem and the seedling soon wilts. When the plants become larger, leaf-feeding tends to supplant stem-feeding, and furthermore, the stem soon becomes large enough to withstand the injury that may occur there. It follows then, that the early appearance of the bug in the field, attacking squash planted late, might cause extensive damage, although in Connecticut this possibility is quite remote. Commercial growers of squash, anxious to

get their product on the early market, either start the squash plants in the greenhouse or plant under hotcaps in the field. In either case the plants bear on the average from 10 to 15 true leaves by the time the squash bug makes its appearance the last of May or early in June. Just how many bugs are necessary to kill a plant of this size (without the aid of other insects or disease) is not known. As many as 80 were caged over such a plant without any appreciable injury resulting. While it is true that under such caged conditions feeding is probably not normal, this far exceeds the usual number found on a single plant in the field at the beginning of the season, even in years of insect abundance.

The sucking mouth parts of the squash bug—typical of hemipteran insects—have been well described by Tower (1914), and the feeding mechanism is similar to that of other Heteroptera as described by Marlatt (1895) and Weber (1930).

In the act of feeding, the bug probes the surface of the plant tissue with the tip of its rostrum. When an apparently suitable spot is found, the stylets are inserted. The insertion of the setae does not necessarily imply an act of feeding. The insect may insert its setae many times before it locates a suitable feeding locus. Histological preparations showing setal paths directed towards vascular bundles and the frequency with which bugs are found with their setae inserted in veins in the leaves suggest that trial punctures are made by the bug in an effort to locate the vascular bundles. Once a suitable spot is found, however, feeding may continue for an extended period. Uninterrupted feeding in one spot for at least an hour has been observed.

The setal path in the stem tissue may appear to be intercellular, but such a condition is merely fortuitous. Normally the setae penetrate directly through the cells. At least in the superficial portions of the path, only those cells directly pierced give evidence of injury. In the deeper portions a more diffuse injury is observable. Whether this is due to a spreading of histolytic substances secreted by the salivary glands or to random mechanical punctures made by the tip of the setae, is not clearly demonstrable.

The photomicrograph (Plate 2, A)* shows a cross-section of squash stem in which a portion of the setal path is evident. The bifurcation suggests that the insect partially withdrew its setae after a period of feeding and reinserted them in another direction. Other sections of this same preparation show more general injury adjacent to the vascular bundle.

Because of the fact that relatively few cells comprise the thickness of a leaf, there is a less definite setal path to be observed in leaf-feeding punctures. Although leaf-feeding may occur on either surface, most of the feeding takes place on the under side. When punctured, the epidermal cells and the palisade cells usually show localized injury. The general injury comparable to that noted above around the vascular bundles in the stem is to be found in the spongy mesophyll of the leaf. This is illustrated in the photomicrograph (Plate 2, B). Probably small veins are usually involved in this mesophyll wounding, but since cytological details are obscured by the injury, they cannot always be distinguished.

* Plates are placed at the end of the bulletin.

Externally the wound early manifests itself as a brown spot due to the drying out of the affected tissue. The size of the spot may vary, depending upon the duration of the feeding and the amount of vascular tissue involved. The wounded areas caused by two or more feeding punctures in close proximity may coalesce, resulting in a still larger brownish patch. Since squash bug nymphs feed gregariously—at least in the early instars—an infested leaf soon assumes a mottled appearance, followed by a complete browning of the affected area. This is the "burning" effect (Plate 2, C) which is typical of squash bug injury.

Ultimately the entire leaf dries up, and with a heavy infestation, the whole plant succumbs. This, it will be noted, is normally not a sudden effect, but the plant dies, as it were, leaf by leaf.

Pack (1930) expressed the belief that the injury to the tissues is due not so much to the removal of plant juices, but to what appears to be a plant disease which is carried by the bugs, and which is transmitted from one generation of insects to the next through the egg. What is far more likely, and the possibility of which Pack admitted, is that toxic or enzymatic substances secreted by the salivary glands and injected into the plant at the time of feeding are chiefly responsible for the injury. This has been suggested by the observations of Robinson and Richards (1931). To date, however, experimental proof of this toxicogenic action is lacking although injury to apple and potato caused by the Capsid bugs *Plesiocoria rugicollus* and *Lygus pabulinus* has been definitely shown by Smith (1920) to be the result of the reaction between the salivary secretion of the insects and the plant tissue.

In the histological preparations made of injured squash tissue, the affected region is always marked by a coagulum which stains heavily with safranin. That this is due to the salivary secretion of the bug or to a product arising from the reaction between this secretion and the plant juices is suggested by the fact that a smear of plant juice fails to stain with safranin, whereas a smear of salivary gland alone or in combination with plant juice does take the stain. This coagulum resembles the stylet sheath of *Aphis rumicis* as described by Davidson (1923), although it is much broader in extent, as would be expected from a much larger insect. The sylet sheath, a reaction product of the saliva and the plant juice, is considered by Davidson to be composed of pectic substances. Callose and insoluble calcium pectate are suggested by the works of Petri (1909) on *Phylloxera vestatrix*, but Davidson believes that the exact chemical varies with the insect species*.

Attempts to simulate injury to squash tissue by means of extracts of the salivary glands of *A. tristis* have given only negative results. Hoerner (1938) injected, hypodermically, both aqueous and saline extracts of the glands into squash plant tissue with no observable results. The writer, using a 30 percent glycerine extract of the salivary glands, followed the technique which Parr (1939) has used so successfully in simulating the injury caused by *Matsucoccus gallicolus* Morr., a gall-forming scale insect on pine. No detectable injury other than that caused mechanically was observed. Similarly, glandular tissue itself, scratched into the surface of

* Carter (1939) has recently reviewed the work on plant injuries caused by suctorial insects.

the leaves and stem of squash plants, gave no evidence of enzymatic or toxic effects. It is quite probable that microchemical tests might indicate injurious effects not otherwise observable.

Suspicion has rested upon the squash bug as a possible vector in the transmission of *Bacillus tracheiphilus*, the bacterium responsible for the wilt disease common to squash. Rand and Enlows (1916) obtained only negative results in a series of tests, and concluded that the striped cucumber beetle (*Diabrotica vittata* Fabr.) and the spotted cucumber beetle (*D. duodecimpunctata* Fabr.) are probably the only agents involved.

Natural Enemies

Bacteria

Duggar (1895) described a bacterium (*Bacillus entomotoxicon*) which may cause an epizootic disease among squash bugs. The prevalence of this disease is not known, and no positive evidence of it was encountered in the present study. Occasionally a dead bug was found which gave evidence of bacterial invasion, and, in fact, showed symptoms of the disease described by Duggar. Extracts of these bugs, however, sprayed upon living healthy bugs failed to result in the death of the latter. Since this was essentially the technique used in some of Duggar's inoculation experiments, it may be concluded that the bacteria in question were not of a pathogenic nature, but merely saprophytic forms attending the death of the insect.

Fungi

Chittenden (1899) observed an entomogenous fungus which appeared on the antenna of a squash bug. The entire body of this bug was pervaded by the mycelium of the fungus which was identified as " 'probably a conidial stage of some Cordyceps, a *Sporotrichum* sp.' ".

Insects

No egg predators have been observed by the writer, although frequently eggs appear to have been damaged by feeding. Girault (1915), in Virginia, observed an adult locustid feeding upon the eggs of the squash bug.

Four hymenopterous egg parasites have been recorded. Chittenden (1899) reared *Hadronotus anasae* Ashm. (Scelionidae) and *Oencyrtus anasae* Ashm. (Encyrtidae) from squash bug eggs and commented on the fact that 30 percent of the eggs collected in Florida by Ashmead were parasitized by *Hadronotus*. Two other species of *Hadronotus* are also known to parasitize the eggs of *A. tristis*. These are *H. ajax* Gir. (Girault, 1920) and *H. carinatifrons* Ashm. (Girault, 1904, 1913). None of these parasites has ever been reported from Connecticut.

Undoubtedly the squash bug population suffers some loss, small though it may be, due to predaceous insects. During the summer of 1935 specimens of the spined soldier bug, *Podisus maculiventris* Say, were found frequently on squash plants, often feeding on squash bug nymphs. In the laboratory several individuals were reared to maturity on a diet consisting almost exclusively of squash bug nymphs. No records were kept of the number of young squash bugs thus destroyed, and the adult Pentatomids were not retained for such observation. During the seasons of 1936 and 1937 only occasional specimens of *P. maculiventris* were observed, and in 1938 none

was encountered on squash plantings. Squash bug nymphs have also been observed to serve as food for the larvae of the lace wing (*Chrysops* sp.), the predaceous bug, *Nabis ferus* Linn., and a spider, *Misumenops asperatus* Hentz. Ants have been observed to feed on squash bugs, but in these cases evidence seems to favor the view that the bugs had been killed or injured by some other agency and that healthy bugs are seldom if ever attacked. The incidence of these predators in the field is too low to be of much importance in the economy of the squash bug population.

Uhler (1876) observed that the squash bug nymphs "are often guilty of cannibalism; the stronger ones sucking the juice of the weaker, and leaving their dried empty skins to attest their places upon the squash vines".* Provancher (1886) also reported this carnivorous tendency. No evidence whatever was observed by the writer, nor by Chittenden (1899) who did, however, observe a squash bug nymph with its beak inserted in a dead nymph of *Leptoglossus oppositus*.

By far the most important insect enemy of the squash bug is a dipterous parasite, *Trichopoda pennipes* Fabr. (Tachinidae). A detailed account of this will be given later.

Vertebrates

Amphibia, reptiles, and birds must be considered as possible vertebrate predators of the squash bug.

Kirkland (1897), in a study of the contents of 149 stomachs of the American toad (*Bufo lentiginosus americanus* LeC.=*B. americanus* Holb.), found specimens of *A. tristis*, but noted that the number of Hemiptera and Diptera together do not form 1 percent of the whole food. Glover (1877) mentioned the feeding on bugs by toads. Judd (vide Chittenden, 1899, p. 26) also noted the presence of a squash bug in the stomach contents of a toad. Conradi (1904), in a study made during a year when the squash bug was unusually abundant, found the contents of 152 toad stomachs to contain less than 3 percent of hemipterous material. This author stated that toads in confinement will eat squash bugs, a fact confirmed in the present study. In the field, predation by toads is probably very limited, and the effect on the bug population might be positive as well as negative, because of the possibility of toads feeding on the parasite of the bug, *T. pennipes*, which also is available in considerable numbers.

Chittenden (1899) reported that a tame lizard, *Sceloporous undulatus*, fed upon squash bugs in spite of their odor, but "one bug . . . sufficed for a meal". This reptile does not form a part of the ecology of a squash planting—at least in Connecticut—and in fact, even insectivorous snakes do not frequent fields of squash.

Bird predation, also, seems to be of negligible importance. The squash bug is not the preferred food of any bird, and in fact, few birds are known to feed on it at all. The food of a number of insectivorous birds includes many Hemiptera, but members of the family Pentatomidae are preferred to those of the family Coreidae. Beal (et al, 1916) recorded squash bugs being found in the stomach contents of the yellow-billed cuckoo (*Coccyzus americanus*), the night-hawk (*Chordeiles virginianus*), and the scissor-tailed flycatcher (*Muscivora forficata*). In the stomachs of several species

* It is probable that this report of Uhler is an exaggeration, and that the "dried empty skins" were nothing more than the exuviae, which are usually to be found in abundance.

of vireos Chapin (1925) found the remains of a number of Coreid bugs, including members of the genus *Anasa*, although no specimens were identified as *A. tristis*. In December, 1937, the stomach contents of four starlings (*Sturnus vulgaris*), examined by students, were each found to contain bug remains which were indentified as those of *A. tristis*. This is significant in that at that time the bugs were hibernating and must have been in quarters accessible to the birds. Kalmbach (1928) found that Hemiptera constitute less than 1 percent of the yearly food of the adult starling. Another example, which has little significance in considering natural conditions, is the case of a tame duck which repeatedly ate squash bugs offered to it.

A very interesting phenomenon was brought to light by the work of Conradi (1901) in regard to the secretion of the repugnatorial glands of the squash bug. Often structures or functions are assigned protective value, falsely based on anthropomorphic interpretations. In this case, however, the odor emitted by the bug has at least potential, if not actual, protective value.

This odor is one of the best known characteristics of the squash bug, and has been described as that of ripe pears. In extreme dilution it is not unpleasant, but in concentration is quite nauseating. The secretion is elaborated by two glands (Moody, 1930), which, in the nymphs, are located just beneath the dorsal wall of the abdomen and communicate to the outside through two apertures, located between the fourth and fifth, and between the fifth and sixth, abdominal segments. In the adult the glands are located in the metathorax and empty by ducts into a common median reservoir which in turn opens externally through two ostioles, one on each side of the body between the meso- and meta-thoracic coxae. The secretion may be ejected with some force, the maximum distance to which the liquid may be thrown being five inches, as found by Moody. This forceable discharge is scarcely necessary, for the fluid is exceedingly volatile, and diffusion of the odor is so rapid that the effect of the nymph, which ejects the fluid with much less force, is fully as great as that of an adult.

The chemistry of this secretion is not definitely known. Verrill (1868) stated that the odor "bore the most resemblance to the formate of the oxide of anyl, or the formate of anylic ether" and that "it is probable that this substance is its most essential and active ingredient". Whether this statement was based on a chemical analysis or merely a comparison of odors was not noted. Certainly the odor does suggest an essential oil or an ester of a higher fatty acid. A steam distillate of bugs has the characteristic odor, but if the active principle is present, it is entirely miscible in water, for no oil separates out.

Conradi (1901) erroneously believed that the odor is due to anal secretion, but his observations on its physiological effect are of real interest. He found that if a squash bug nymph was dropped into a wide-mouthed bottle containing a toad, the repugnatorial secretion exerted an anaesthetic action by throwing the toad into a temporary stupor. The effect on the toad seemed to be directly proportional to the concentration of the vapor (i.e., the number of nymphs dropped into the bottle). Moreover, if sufficient bugs were introduced, the toad succumbed. This observation was confirmed by the writer. Conradi noted that red-spotted salamanders

and frogs responded as did toads, although more bugs might be necessary to produce the effect. No ill effects could be observed on snakes. The writer subjected a Japanese salamander (*Triturus pyrrhogaster*) to the vapor, and death resulted as with the toads.

A steam distillate of bugs was tested for toxic effect by enclosing a frog with some of the fluid in a closed jar. No effect on the frog was observed, nor on a newt (*Triturus viridescens*) which was similarly treated. Injection of the fluid into the dorsal lymph sac of the frog likewise produced no stupor.

Both Conradi (1904) and Girault (1912) made sketchy observations on the effect of bug odors on other insects. Various stink-bugs (Pentatomidae) were used in addition to squash bugs, but the results obtained were highly variable. In a few cases noxious effects were observed, but definite conclusions could not be drawn.

II. BEHAVIOR AND BIONOMICS

ALTHOUGH several generations of *A. tristis* occur each year in the southern states, in Connecticut mating and oviposition do not normally take place during the same season in which the bugs mature, but are delayed until the following spring. A few exceptions to this were noted in 1938, when bugs maturing the first few days of August deposited eggs after the short pre-oviposition period of but two days.* This was not noticed during the other five years in which the bug was under observation. When kept under conditions of maintained high temperature and humidity in the laboratory, female bugs may mate and deposit fertile eggs within a few weeks after becoming adults. This reponse is not uniform, and many individuals resist all attempts to break the resting condition of their reproductive organs. Preliminary experiments indicate that the individual factors of temperature, humidity, and light intensity, either with or without the bugs being subjected to an intervening period of dormancy, are ineffective. At present facilities are not available for the satisfactory testing of environmental factors in combination which might break the reproductive diapause.

When the female bugs first appear in the field in the spring their ovaries are in the hibernating condition, but development is rapid and oviposition begins within one or two weeks.

The first appearance of *A. tristis*, as observed by the writer in squash fields in the vicinity of New Haven, is as follows:

1935	June 15
1936	May 25
1937	June 7
1938	June 2
1939	May 31

* The oviposition of these bugs closely paralleled that of the few remaining overwintered bugs, ending at the same time in early September. This indicates that the curtailment of reproductive activity is not inherent in the reproductive organs of the insect, but is due to the impingement of external environmental factors.

Mating

Mating of squash bugs takes place before they appear in the field, or immediately after, for no female taken in the field in early spring has been found to be sterile. Even the first females observed, if segregated, lay fertile eggs. Throughout the duration of the reproductive season of the bug, copulation occurs frequently and for prolonged periods of time. This multiple mating is not essential for continued deposition of fertile eggs, as was shown in 1938. Ten of the first females observed in the spring were permitted one observed copulation and were then segregated in individual cages[1] in the field. Such segregation resulted in no loss of fertility, and the oviposition records of these 10 bugs can be compared with the records of 54 other females similarly caged, but provided with males. The following figures indicate the average number of eggs deposited per female throughout the season and the average number of days survived by each female after the date of caging:

	Average number of eggs deposited	Average longevity in days
Females with males	181 ± 10.4	43 ± 1.9
Segregated females	308 ± 21.3	81 ± 8.4

These figures[2] indicate that segregated females live longer and lay more eggs than females which copulate promiscuously. In nature, however, this behavior has little significance, for both sexes are polygamous and are always present in about equal numbers.

Sex Ratio

Girault (1904), knowing that the male of the squash bug is polygamous, believed that it was to be found in relatively fewer numbers. A collection of 1000 bugs in August and September, 1935, showed 499 males and 501 females, indicating that the newly matured bugs have a sex ratio of .5. Moreover, no evidence was obtained to indicate that a differential mortality exists to alter this ratio throughout the year. Tabulations made during June and July, 1936, of 1430 overwintered bugs, gathered at random in the field, included 653 males and 677 females, the difference being of no significance as tested by the chi-square.

Oviposition

The number of eggs deposited in each cluster is highly variable, with single eggs being found occasionally, and the maximum number so far observed in these studies being 47. Of 1000 egg masses gathered at random from the field, the mean number per mass was found to be 14.2 ± .15 eggs.

Hourly Oviposition

Although the oviposition of *A. tristis* is not restricted to any particular time of day, it tends to be concen-

[1] The cages used were devised (Beard, 1937) for use on plants growing in the field. A vigorous squash plant can accommodate a number of these cages with very little injury to the plant. Consequently this method is far more convenient than growing potted squash plants for individual caging.

[2] The differences between the means have high significance statistically (Student's t-test giving a value beyond the 1 percent level). Throughout this study, the statistical methods described by Snedecor, 1938, have been followed. It will be shown below that a close correlation exists between longevity and the number of eggs deposited. In the above, it would seem that the effect is on longevity, the increased egg production being merely a manifestation of this.

trated in the hours of higher temperatures. This was shown by observations made July 14, 1936 and July 16, 1937, when oviposition was at the seasons' peaks and the weather was clear and hot. The egg deposition was noted hourly from daylight until darkness. On both days, 64 percent of the eggs were deposited between the hours of 11 a.m. and 3 p.m. Some oviposition does occur at night, and females confined in darkness continue to lay eggs. Thus light intensity seems a less significant factor than temperature.

Daily Oviposition The day-to-day oviposition was recorded during the 1936 season, when the eggs deposited on 36 hills of summer squash were gathered daily. The egg counts were made at as near the same time each day as possible, and weather records were obtained for the time interval between counts. The period of observation included a total of 95 days, beginning when the first eggs were deposited on June 6 and ending when oviposition ceased on September 9. The egg masses collected number 1215, containing a total of 17,194 eggs. The number of eggs deposited per day varied widely, ranging from 0 to 535. Assuming that the total population fluctuates but little from day to day even though the seasonal change is great, the variations can best be explained by weather conditions. When the counts were made during the summer, it seemed that the greatest number of eggs were laid on the hottest days. Accordingly, the correlation coefficients of oviposition and the environmental factors of temperature and humidity were calculated as follows:

		r =
Oviposition and:	Minimum temperature	.191
	Maximum temperature	.259
	Mean temperature	.277
	Minimum humidity	−.155
	Maximum humidity	−.121
	Mean humidity	−.208

Although the correlation coefficient is used advisedly because of the seasonal fluctuation of the total population of reproducing bugs, the figures indicate that the mean temperature and oviposition have a highly significant correlation (beyond the 1 percent level of significance). The coefficients for maximum temperature and mean humidity lie between the 1 percent and 5 percent levels of significance; the correlation coefficients for the other factors have little significance. A very low correlation is to be expected with maximum humidity, which varied but little below 100 percent.

Conversely, cool, cloudy, and rainy weather retard oviposition. The question that arises is whether the egg production itself as well as the oviposition is retarded, or whether the increased oviposition on warmer days compensates for the apparent loss in egg production. This can be determined from the oviposition records of individual bugs by considering the number of eggs deposited following intervals during which no oviposition occurs. If egg production proceeds at a uniform rate, the oviposition during longer units of time should be the same, in spite of wide daily fluctuations. If, on the other hand, oviposition is a direct expression of

egg production, any inhibiting influence may be interpreted as causing an actual decrease in the total number of eggs deposited. In the following analysis, 627 egg masses from 45 squash bugs are considered. The tabulations below indicate the average number of eggs deposited by a bug before and after intervals of increasing length during which no eggs were deposited.

Although the largest number of cases suggest that a bug may deposit eggs every day, it is rare that oviposition occurs on more than six successive days.

AVERAGE SIZE OF EGG MASS BEFORE AND AFTER INTERVALS WITHOUT OVIPOSITION

Days interval without oviposition	Average number of eggs per mass		Number of cases
	Before interval	After interval	
0	13.5 ± .36	10.7 ± .29	238
1	15.1 ± .45	14.3 ± .31	179
2	15.4 ± .59	15.1 ± .60	81
3	15.3 ± 1.09	16.9 ± 1.03	52
4	15.5 ± 1.17	18.0 ± 1.16	30
5	12.9 ± 1.20	20.8 ± 1.69	19
6	13.5 ± 1.20	20.0 ± 1.35	16
7	10.3 ± 1.00	17.2 ± 2.42	13

No periodicity was detected in the oviposition of individual bugs, and the above data clearly show no correlation between the size of the egg mass and the interval to follow. On the other hand, the size of the egg mass after an intervening period with no oviposition does tend to increase as the length of the interval increases. The largest average size of the egg mass appears to follow a five-day interval, but the number of cases of the larger intervals is too limited to conclude definitely that the peak is reached at this time. This increase suggests but a limited compensation, and it is obvious that more frequent oviposition results in a larger total egg production, even though the number of eggs deposited at one time is smaller. Clearly, then, periods of limited oviposition are due to restricted reproductive activity and not to a mere withholding of the eggs until conditions are favorable for their deposition.

Seasonal oviposition

The oviposition trends for 1934*, 1935, 1936, 1937, and 1938 were determined by collecting at weekly intervals all the squash bug eggs present on certain hills of summer squash. Although with one exception (1937) more than 10 hills were under observation, the data presented in the chart (Figure 2) were calculated on the basis of number of eggs per 10 hills of squash. The trend of each season is thus comparable with the others. The curves for 1935 and 1937 are practically normal, with the peak of egg production occurring during the second week in July. Single peaks of oviposition also occurred in 1934 and 1938, but they appeared earlier in the season. The 1934* data are incomplete, as observations were begun after the

* Data of Elliott (1935).

beginning of oviposition. The very early peak observed in 1938 and the subsequent low rate of oviposition throughout the remainder of the season is easily explained on the basis of population density, to be discussed below. The 1936 trend shows three modes, the major one coinciding in time with the peaks observed in 1935 and 1937. The cause of the fluctuations, producing the tri-modal curve, is not apparent. Since the oviposition shows no correlation with the mean temperatures of the periods under consideration, it is very likely that fluctuations in the adult population were responsible. The 1936 oviposition was also peculiar in that it began two weeks earlier and ended two weeks later than that of other years. Thus the season was fully a month longer.

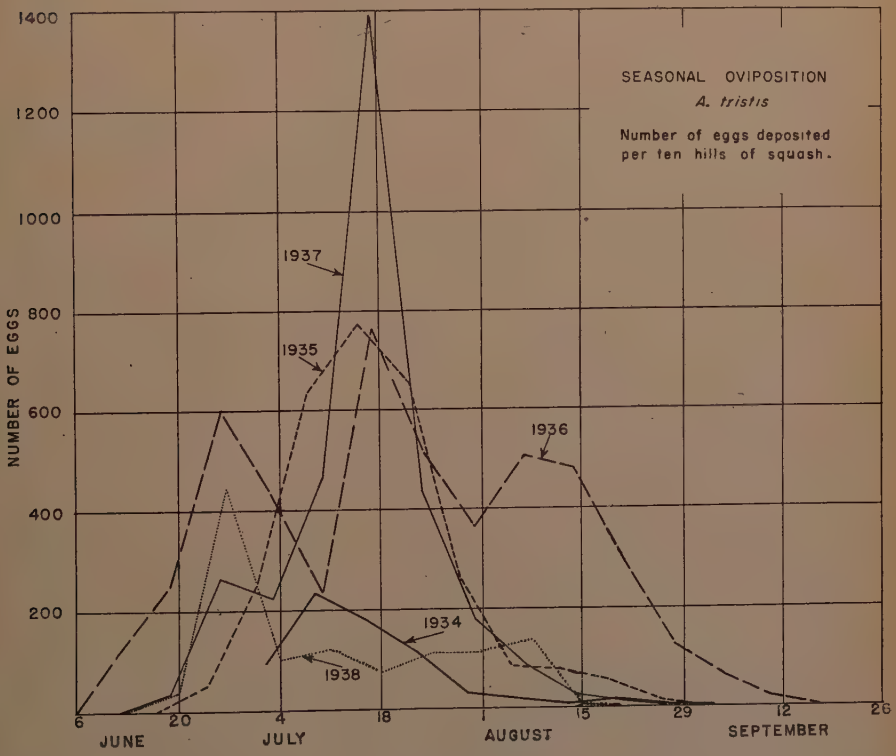

FIGURE 2. Seasonal oviposition *A. tristis*. Number of eggs deposited per ten hills of squash.

The same data on seasonal oviposition are presented in a different form in Figure 3, in which the percentages of the total number of eggs deposited are plotted against time. In other words, the cumulative oviposition throughout the season is considered with reference to the total egg production. Again the similarity of the 1935 and 1937 seasons is evident. The two curves, closely approximating each other, show the expected sigmoid shape. In both cases almost 70 percent of the eggs were deposited during the first three weeks in July, and by the first of August over 90 percent of

the eggs had been deposited. The sigmoid shape is less apparent in the curve representing the 1936 oviposition. The curve of the 1938 oviposition emphasizes the effect of population density. When the population was at its height the last of June, 40 percent of the season's eggs were deposited within the interval of only one week. The remaining 60 percent of the eggs, representing the production of a small population, was deposited over a period of eight weeks.

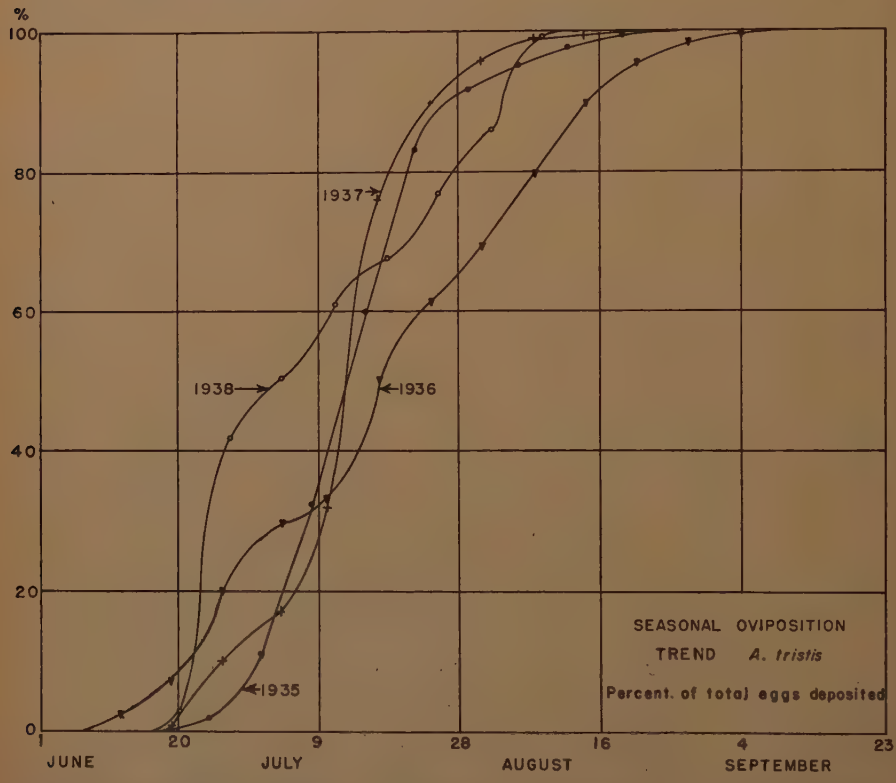

FIGURE 3. Seasonal oviposition trend *A. tristis*. Percentage of total eggs deposited.

In spite of the two secondary modes observed in 1936, there is a tendency for the oviposition in the field to increase up to a certain point and then decline. If the fecundity of individual bugs followed a similar trend, a summation of the reproductive activity of all the bugs comprising a stable population would produce such a seasonal trend. On the other hand, if the fecundity of the individuals remained uniform throughout the season, an increase and subsequent decline in the total population of reproducing bugs could account for the trend. If, however, the individual fecundity and the population density vary and interact, either a cumulative or a compensatory result might be expected. In the former, the peak of oviposition would be accentuated. In the latter, a large population could compensate for low fecundity (or vice versa), obscuring the peak and resulting in a more uniform oviposition.

Several considerations point to the conclusion that population density is the most important factor responsible for the peak in the oviposition trend of the season. In the first place the peak oviposition in 1938 and 1939 coincided with the maximum population density, which was closely observed. A comparison of the two trends for 1938 (Figure 4) is here shown. The sharp rise in the early oviposition indicated the onset of a heavy infestation, which fact was substantiated by counts of adult bugs. During the last few days in June, however, an unusually heavy rainfall caused a marked depletion of adult bugs. The sudden drop in oviposition reflects this loss, and the uniform egg production during the remainder of the season was maintained by a few surviving insects.

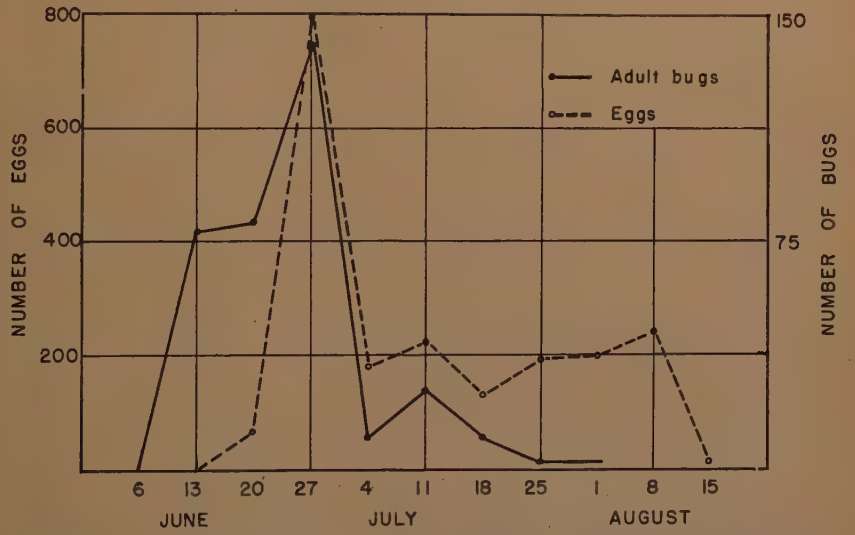

FIGURE 4. Chart showing comparison of population density with oviposition of squash bug.

The conclusion that population density chiefly determines the trend of oviposition is further supported by a comparison of the seasonal oviposition trend with the fecundity trend of individual bugs. This can best be done from data obtained during 1937, which is illustrated by the chart, Figure 5. The seasonal oviposition trend, represented in percentages of the total number of eggs deposited (A), shows the same sigmoid curve figured above. For the fecundity trends of bugs caged individually in the field, two sets of data are given. The reason for this lies in the fact that very few bugs deposit their full complement of eggs, but die before their reproductive organs cease activity. Accordingly, one set of data (C) includes the oviposition records of three bugs which did lay eggs throughout the season until their ovaries became inactive. The other set of data (B) includes the records of 10 bugs which died during the first weeks in August, when the rate of mortality was the highest. In both cases the fecundity trend is seen to proceed at a much more uniform rate than the seasonal oviposition trend. In neither case is there an accelerated fecundity rate

coincident in time with the accelerated oviposition of the whole population, indicating that the peak oviposition is not due to increased fecundity.

In view of the uniform fecundity trend of individuals, the number of eggs deposited should be a rectilinear function of the longevity of the bug.* This is actually the case, as can be seen in Figure 6, which represents the oviposition records and longevity in days (following the time of appearance in the field after hibernation) of 101 female squash bugs. Each bug, with a male, was retained in an individual field cage. The observations included both 1937 and 1938 seasons. The correlation coefficient of oviposition and longevity is .872, which is highly significant (beyond the 1 percent level). The regression coefficient of the data here plotted is 4.7.

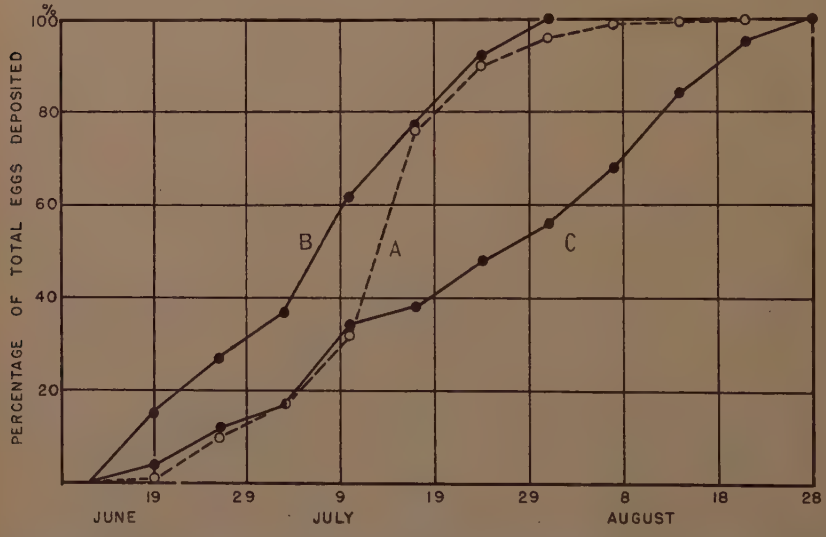

FIGURE 5. Chart showing cumulative oviposition trend of total population (A), ten bugs which died during the first week in August (B), and three bugs which completed their oviposition (C). 1937.

Reproductive Potential

The question now arises as to what can be considered the reproductive potential of the squash bug. The data on longevity reported above suggest that the number of eggs deposited by a bug is limited more by environmental conditions than by the number of cells in the ovaries potentially capable of differentiating into ova. Pertinent to this problem, the following experiment was designed to test whether or not one ovary in a bug could compensate for the loss of the other by producing more than one-half the number of eggs produced by a normal bug.

The oviposition record of a series of bugs subjected to surgical castration of one ovary was obtained and compared with a series of normal bugs.

* If based on seasonal averages. It was shown above that weather conditions may modify oviposition if short units of time alone are considered.

The operations* were made on the first females to appear in the field in June, 1938, when the ovaries were still in a rudimentary condition. Of 12 such operations, 11 were successful. One of these operated bugs was lost early and so cannot be included in the analysis. Each of the remaining 10 was retained, with a male bug, in an individual field cage. The 54 normal females used for comparison were similarly caged.

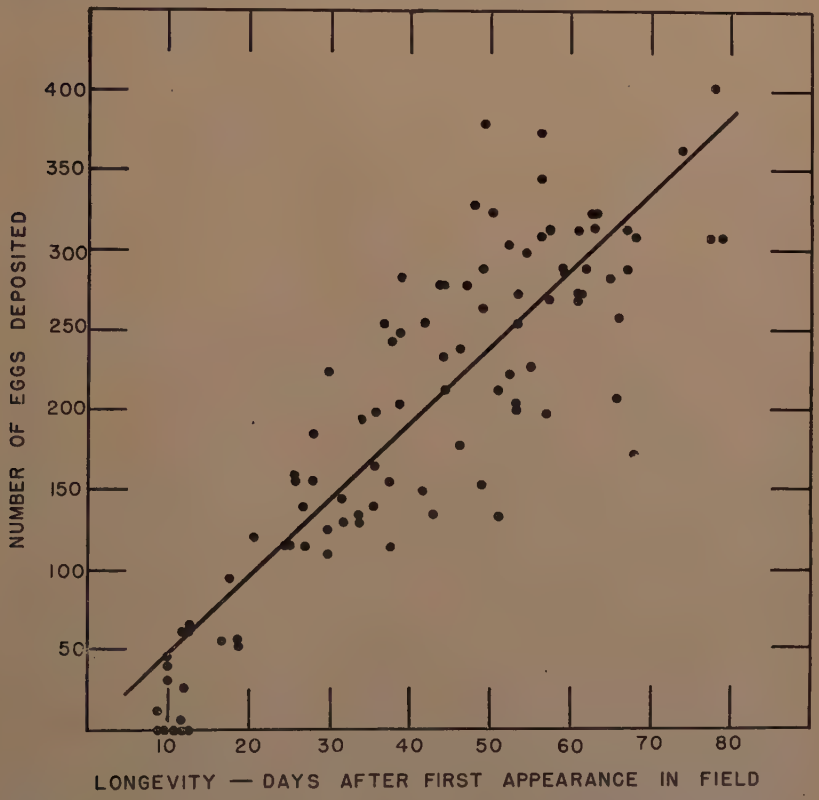

FIGURE 6. Chart showing number of eggs deposited in relation to the longevity of the bug.

The duration of life of the operated animals varied from 20 to 69 days after the operation, with an average of 45.7 days. The normal bugs lived from 14 to 111 days (after June 8), with an average of 47.2 days. (If one bug which lived 111 days is excluded, 71 days is the maximum, and 46.0 the average.) Since the duration of life of the operated animals falls within range of the normal, and the averages are not significantly different, it is evident that the operation itself had no effect on the longevity of the bugs.

Upon the death of each experimental bug, dissection was made to verify the success of the operation. In all 10 cases the autopsies showed that the

* A description of the technique used in these operations is deferred to a later chapter. See appendix.

castration of one ovary was complete and that unquestionably all the eggs produced had developed in the intact ovary.

In regard to the number of eggs deposited, it was found that the experimental insects laid an average of 119.9 eggs, 66.3 percent of the average laid by normal bugs, which was 180.8 eggs. These figures suggest that one ovary cannot make up entirely for the loss of the other, but there is compensation amounting to somewhat more than 30 percent. A more satisfactory analysis can be made if each operated animal is considered individually.

In the following table, comparison is made between the operated bugs and the normal bugs which were living at the time of death of the experimental bugs. In other words, the oviposition of the operated bugs is compared with the oviposition of the normal bugs during the same time interval.

OVIPOSITION OF UNI-LATERALLY CASTRATED BUGS COMPARED WITH THAT OF NORMAL BUGS

Operated Bugs			Normal Bugs				
			Number of eggs deposited				
Case number	Days survived after operation	Number of eggs deposited	Min.	Max.	Mean	Mean / 2	Number of bugs
1	20	12	0	122	52	26	44
2	27	32	0	161	77	39	40
3	27	54	0	161	77	39	40
4	34	63	26	214	117	59	39
5	39	129	50	265	150	75	37
6	49	141	100	304	195	98	30
7	63	195	158	318	260	130	13
8	64	153	167	318	268	134	13
9	65	149	173	318	273	137	11
10	69	271	176	338	254	127	5

From these data it may be observed that: In all but two cases (numbers 8 and 9) the number of eggs laid by the operated bugs exceeds the minimum number laid by the normal bugs. In no case does the number exceed the normal maximum. In one case (number 10) the number exceeds the mean of the normal. In all but two cases (numbers 1 and 2) the number of eggs deposited by the operated bugs well exceeds one-half the mean of the number of eggs deposited by normal bugs. With the exception of number 10, compensation by the intact ovary cannot be considered complete. Nevertheless it is clear that in the absence of one ovary, the remaining one may produce more eggs than it would otherwise. Consequently, the reproductive potential, implying the theoretical number of eggs the insect is potentially capable of producing, is quite certainly never realized. This is but further evidence of the well-known view that the numerical abundance of many species of insects depends to a very limited extent upon their powers of reproduction and almost entirely upon the factors which tend to limit these powers. The term "reproductive capacity" rather than

"reproductive potential" should be used as an estimate of the average number of eggs the bug will actually deposit under a given set of conditions.

As to what this average might be numerically, Girault's (1904) estimate of 154, based upon observation of a single female, seems much too low a figure. Wadley (1920) found that in 1917 overwintered females deposited on the average 502.5 eggs each, and in 1918, 419. He did not indicate, however, the number of bugs from which the average was determined nor the maximum number of eggs deposited by any one female. From 142 female squash bugs kept under observation during 1937 and 1938, a total of 34,280 eggs was obtained. The number deposited by each individual ranged from 0 to 836, with a mean of 241.4 ± 9.5.

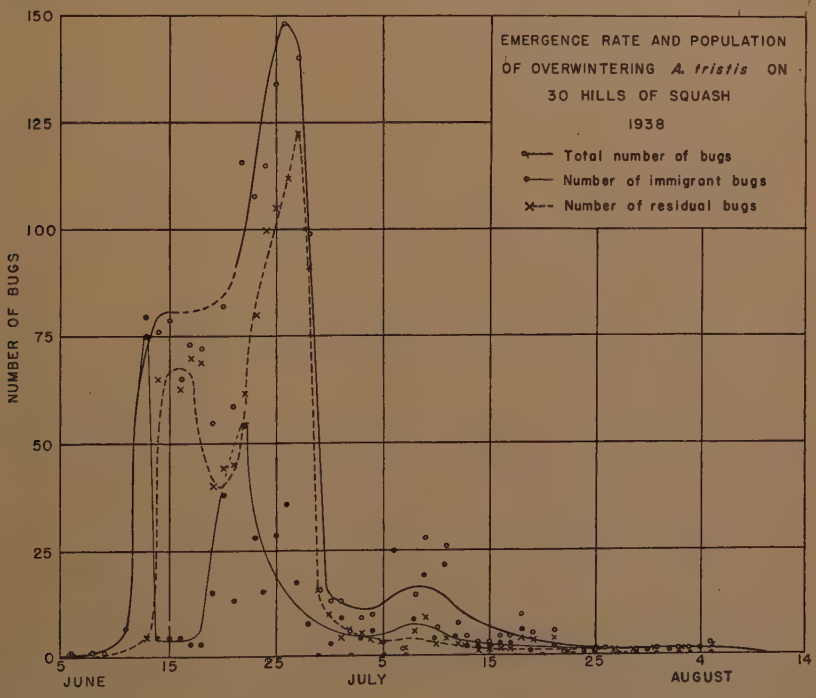

FIGURE 7. Emergence rate and population of overwintering *A. tristis* on 30 hills of squash.

Emergence Rate and Population Density of Overwintered Squash Bugs

Although the work reported above indicates that the oviposition trend serves as a good index of the numerical abundance of overwintered *A. tristis*, actual counts of these adult insects were obtained for 1938 and 1939 by the use of trap boards. It has long been known that squash bugs tend to congregate at night under loose boards placed under the food plants. Advantage has been taken of this tendency by thus trapping and collecting the bugs as a control measure. Accordingly, shingles were placed at the

base of each hill of summer squash within a single plot. Each morning, before they became very active, the bugs under and in the immediate vicinity of each shingle were counted, and each insect was marked with yellow enamel. Thus the presence of this mark indicated that a bug had previously been observed, and so the residual bugs could easily be distinguished from those which newly entered the squash planting.

Data for 1938 are shown in the accompanying chart (Figure 7). Considerable daily variation of both the residual and immigrant bugs was noted, and the presence of marked bugs in plots of squash at some distance from the observational plot indicated some migration from one area to another. The total population counts followed a more definite trend, indicating that the migration was not uni-directional, but entirely at random. Moreover, the curves of the total number of bugs and the number of residual bugs are roughly parallel. Because of the migration, the number of immigrant bugs does not represent only those actually emerging from hibernation. Even so, two peaks are noted in the curve representing the number of immigrant bugs. The first is most striking, for in one day (June 13) 75 new bugs appeared. These certainly represented emergents from hibernation, for very few bugs were present prior to that time. The peak of the total population was reached on June 26, when 148 insects were noted. On this and the two days following, however, an unusually heavy rain fell (4.33 inches), with the interesting result that 90 percent of the bug population disappeared. This phenomenon seemed to be an actual exodus of the insects rather than a destruction in place, for very few dead bodies were observed. But, since no return migration increased the population, there is no doubt that most of the bugs were destroyed—either in the field or en route to places more sheltered from the rain. This sudden disappearance also had a marked effect upon the abundance of the squash bug parasite, *Trichopoda pennipes*. This will be discussed later. Except for a more gradual decline in the population, the trend for 1939 did not differ significantly from that of 1938.

Mortality

Mortality of Overwintered Bugs

Such a sudden disappearance of the bug as occurred in 1938 is not normal, but judging from the oviposition trends and from the 1939 data, the population of the reproducing adults reaches a peak early in July to decline thereafter. By the middle of August it is difficult to find any old bugs in the field. A few, of course, may remain alive for several weeks, but as the new generation matures, the old bugs form a decreasing proportion of the total population.

The rate of decline of a known population of 122 bugs is illustrated by the accompanying chart (Figure 8). The bugs here represented were retained in the small field cages. Since all were protected from parasitization and predation, the mortality is due to other natural causes. Inasmuch as all of the bugs were collected at the first of the season, the population starts at its peak, thereby differing from a natural field population which builds up to a peak as the bugs emerge from hibernation. Nevertheless, a gradual decline is apparent. Early in August the rate of decline is slightly accelerated, and by the middle of August the population is reduced to a very small number. One of these remaining few survived until November 3.

Egg Mortality The mortality of *A. tristis* in the egg stage is very low. Elliott (1935) noted that of 984 eggs, 96.1 percent hatched. In 1936 the writer observed that of 20,991 eggs, 96.7 percent hatched, and in 1938, 94.6 percent of 1000 eggs produced nymphs. Combining these figures, a mortality of only 3.5 percent is observed. All of these eggs were kept in Petri dishes, but it is doubtful if the mortality of eggs in the field exceeds this figure. It proved difficult to test this due to the fact that at the time of hatching or possibly before, the eggs or the empty shells are likely to be dislodged from the leaf surface to which they are attached. This dislodgment cannot be correlated with rapid leaf growth. Since it usually occurs within a day of the time of hatching, it probably is due to movement involved in the process of hatching, or to a drying of the adhesive substance by which the egg is affixed. In any case, the loss of eggs from the leaf does not necessarily prevent their hatching. For this reason eggs escaping observation by dislodgment cannot be numbered with those definitely known to fail to hatch. The only field data obtained were of 1454 eggs, of which 171 were definitely known to have hatched. The fate of the remaining 1283 eggs escaped observation.

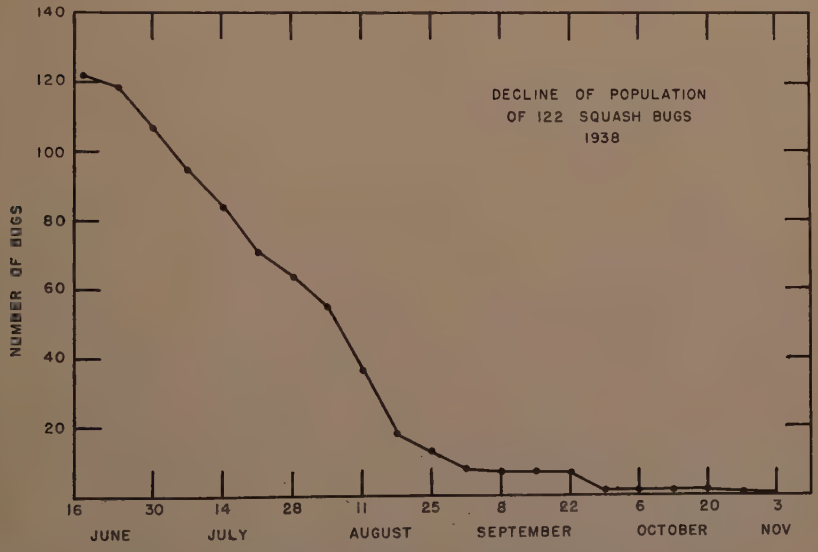

FIGURE 8. Decline of population of 122 squash bugs. 1938.

Nymphal Mortality Due to the length of time required for development and to the extended oviposition period, the possibility exists for the failure of some of the bugs to complete their development in time to seek hibernation quarters in the fall. Of the five seasons observed by the writer, in only one—1936—was this possibility realized. It was shown above that the oviposition period of this season extended two weeks longer than that of the other years. Observations on nymphs developing from the last eggs deposited, and calculations based on the number of eggs produced, led to the estimate that approximately 3 percent of the total population failed to reach maturity in time to find winter shelter.

The total nymphal mortality is even more difficult to determine than the egg mortality. Data of caged nymphs are of no value, for the insects do not feed normally under cage conditions. The best data were obtained by enumerating the bugs which matured on particular hills of squash isolated from adjacent plants. In 1938, on four hills, a total of only 119 adult bugs were obtained from 1454 eggs. Due to the high hatchability of the eggs, it is reasonable to assume that the bulk of this high mortality (91.8 percent) occurred in the nymphal stages. In 1939, 21 hills of squash were similarly observed. From 5274 eggs, 663 bugs reached maturity, indicating a mortality of 87.4 percent.

A different approach to this problem was made in 1937 by counting at weekly intervals all stages of the insect. This was found to be unsatisfactory in estimating mortality, but at least 10 percent of the eggs were known to result in mature bugs.

Hibernation

The flight of *A. tristis* from the squash field to sheltered places for hibernation occurs at no particular time, but extends throughout the month of September. It is particularly apparent on clear warm days, when at frequent intervals bugs individually take to flight and continue in a direct line as far as the eye can follow. The direction of flight, as far as can be determined observationally, is entirely at random. No communal response, either of a temporal or a directional nature, can be noted in the migration. This is borne out by the fact that in hibernation quarters themselves the bugs are not found congregated in numbers, but usually occur singly or in small groups.*

Most general accounts state that the squash bug hibernates under rubbish and debris of various kinds, under loose bark, in crevices, or between boards of buildings. Many hours have been spent in search of hibernating bugs under piles of rubbish of all kinds, in the loose wood of stumps and trees, and under rocks at various distances from squash fields, but no bugs have been found. It is not unusual, on the other hand, to find dormant bugs in buildings. They may occur in the basements of dwellings, or elsewhere in less finished buildings. The insects are commonly wedged in crevices or between boards in response to a positive thigmotropism.

Various hibernation experiments have been conducted in an effort to get estimates of winter mortality. In 1936, of 1300 squash bugs confined in 13 ground cages filled with litter of various degrees of compactness, none survived. In the cages many of the bugs succumbed without getting under the litter provided. This implies that the bugs did not accept the litter as shelter. It is presumed that excess moisture caused the death of those which did get under the litter.

In the fall of 1937, 600 squash bugs were placed in a large cage, six feet square. In one corner of the cage was placed a large pile of loose corn stalks; in the opposite corner was a pile of loose boards. The bugs thus

* Worthley (1923), however, has noted more than 50 bugs hibernating in one location.

had greater freedom of movement and a wider choice of shelter than in the cages employed the previous year. A spring count, made on April 16, 1938, showed 23 insects alive—a survival of 3.08 percent.

Another experiment made in the fall of 1937 consisted of marking 1000 bugs prior to their departure from the field. The mark was a conspicuous spot of yellow enamel placed on the pronotum of each insect. During the following season three of these bugs were recovered—one each on June 23, July 12, and July 17. One bug was reported to the writer on July 15, but since the specimen was not captured, it may have been the same one that was taken July 17. Due to the fact that other marked bugs may have reached other squash fields, these figures are of little value in estimating winter mortality. They indicate, however, that some bugs do return to the same area occupied the previous season, but this number is small due either to a high winter mortality or to a wide scattering and re-distribution.

On October 6, 1938, 150 squash bugs were caged in the insectary with a supply of shingles and loose bark. On February 17, 1939, 84 of the bugs were still alive, indicating a mortality of 44 percent. Although this count was made three months before the bugs should appear in the field, the coldest part of the winter had passed.

Knowlton (1931) observed a 45 percent mortality of bugs caged in an insectary at Logan, Utah, during the winter of 1929-30, but he did not state the conditions provided nor the number of squash bugs tested.

Inasmuch as bugs reach the adult stage at times varying from the first of August until October, the possibility exists for those maturing early to be better or less prepared to survive the succeeding winter than those which mature late.

The minimum lethal temperature (freezing point) was determined for several series of bugs which matured at different times during 1938. It is known that when an insect is subjected to a falling temperature, its internal temperature also falls until the undercooling point is reached, when, due to the heat of crystallization, the body temperature rises to a point termed the rebound point. The technique of Robinson (1927), using a thermocouple and a string galvanometer, was followed in determining the undercooling and rebound points of the squash bug. For each determination the thermo-junction was inserted in the anus of the bug which was then introduced into a chamber cooled by dry ice in alcohol. The undercooling and rebound points can be easily calculated from the deflections on the galvanometer scale. The results tabulated below represent 97 determinations, comprising four series, as indicated:

Date of determination	Date of maturity of bugs	Rebound point C°	Undercooling point C°
Sept. 1	Unknown[1]	−4.2 ± .21	−10.3 ± .45
Sept. 27	Aug. 8–13	−2.8 ± .23	− 7.1 ± .59
Sept. 26	Aug. 22–27	−5.1 ± .18	−12.6 ± .53
Sept. 29	Sept. 4–10	−4.0 ± .26	−10.1 ± .76

Although the undercooling point is to be considered the temperature below which this insect cannot survive, the above figures are higher than

[1] Bugs gathered at random in the field.

the expected minimum winter temperature in this locality. Either the rapidity with which the temperature of the insect is lowered in these determinations results in an usually high reading, or hibernation quarters give sufficient protection against temperature below these if the bugs are to survive.* In any case, no very significant differences in cold resistance can be noted among the four series. Another series of bugs which matured September 18 to 20 was to have been tested, but this was destroyed by the hurricane of September 21.

III. *TRICHOPODA PENNIPES* Fabr., A Parasite of *ANASA TRISTIS*

GENERAL ACCOUNT

Description and Synonomy

TRICHOPODA PENNIPES Fabr. is a very common parasite of members of the families Pentatomidae and Coreidae (Hemiptera). A member of the family Tachinidae, it is a fly of about the size of the common house fly. (Plate 1, D). It has no popular name in common use, but Bratley (1933) referred to it as the "feather-legged fly" because of a prominent fringe of feather-like setae on the outer side of the hind tibia.

In addition to these setae, the brilliant markings of the fly are very conspicuous and render it a most attractive insect. The head and thorax are richly marked with gold and a velvety black. The abdomen is bright orange which darkens at the tip, particularly on the female, where it is shining black. This latter is the most conspicuous secondary sexual characteristic, although the wing of the male bears a ferrugineous spot at its base, along the anterior margin. This spot varies in prominence among individuals, but is seldom as dark as the uniform grayish-black of the wing of the female. These characteristics were early thought to mark different species (see below), and some authors[1] associated them with the wrong sex. Worthley (1924) correctly distinguished the sexes on the basis of these colorations, and also noted a minor difference in the size of the pulvilli, which in the female are smaller and more inconspicuous than in the male.

Fabricius (1794) first described this species as *Musca pennipes*, but later (1805) changed the genus to Dictya. He also described (1805) *Thereva hirtipes*, *Thereva pennipes*, and *Ocyptera ciliata* which have all proved to be synonymous with the species in question. The *Phasia jugatoria* of Say (1829) is likewise a synonym. Latreille (1825) erected the genus *Trichopoda*, into which the *Dictya pennipes* of Fabricius was placed by Wiedemann (1830) and Robineau-Desvoidy (1830). *Trichopoda flavicornis* and *T. haitensis*, described by Robineau-Desvoidy (1830), have fallen as synonyms. Brauer and Bergenstamm (1891) showed that the *T. pyrrhogaster* and *T. ciliata* described by Wiedemann (1830) were but females of *T. pennipes*. This was confirmed by Townsend (1891), who later (1897)

* The possibility that the bugs tested had not yet become cold-hardened is rendered improbable by the fact that four bugs in hibernation (Feb. 20, 1939) were killed at an average undercooling point of only −6.7 degrees C.

[1] Osten-Sacken (in footnote in Say (1869)); Cook (1889); Osborn (1919); Drake (1920).

s ated that the *Thereva pilipes* described by Fabricius (1805) was synonymous with *T. pyrrhogaster* Wd. and might be considered as a variety of *T. pennipes.*

Geographical Distribution

Of the genus *Trichopoda*, which belongs to the fauna of the Americas, *T. pennipes* is the most widely distributed and is the only member of the genus represented on the Pacific Coast (Townsend, 1893). It ranges from New England to the Argentine Republic (Giglio-Tos, 1894), and from the West Indies to California. In the United States it is found in most of the eastern states and in the southern states along the Gulf, being absent in the Northwest, according to Aldrich (1915). Townsend (1893) noted that it is found but little above sea level, although he reported it inland from Michigan, Indiana, Illinois, and Iowa. Coquillett (1897) added to this list Pennsylvania, Mississippi, Kentucky, Missouri, Kansas, and Colorado, as well as Ontario, Canada. Van der Wulp (1888) noted its presence in Mexico and Guatemala. It occurs on islands of the West Indies, including Guadeloupe, Porto Rico, Haiti, St. Vincent (Aldrich, 1905), and St. Croix (Wilson, 1923).

Food

T. pennipes is a nectar loving fly, but in cages will feed on sugar and mixtures of honey and yeast. Townsend (1897) collected *T. pennipes* from the flower of *Cordia* sp. In Florida, Drake (1920) reported having collected the fly on the flowers of Chinquapin (*Castanea pumila*), Basswood (*Tilia americana*), Rattle-box (*Crotalaria usaramoenvis*), Angelica tree (*Aralia spinosa*), Pepper-vine (*Cissus* (*Ampelopsis*) *arborea*), and the Thoroughwort (*Eupatorium* sp.). In Massachusetts, Worthley (1924) noted the feeding on Meadowsweet (*Spirea salicifolia*) and Wild carrot (*Daucus carota*). Drake (1920) detected the presence of sugar in the alimentary tract of flies which had previously fed on the flowers of *Crotalaria*, but he found no pollen grains.

Hosts

Heteropteran hosts of this parasite are to be found in the families Pentatomidae and Coreidae. The reported hosts are here listed:

Host	Authority
Coreidae	
*Acanthocephala declivis Say.	Drake (1920)
*Acanthocephala femorata Fabr.	Osborn (1919), Drake (1920), Watson (1921)
Anasa armigera Say.	Chittenden (1899)
Anasa repetita Heid.	
Anasa tristis DeG.	see below
*Archimerus calcarator Fabr.	Drake (1920)
*Corecoris (Spartocera) batatas F.	Dozier (1926), Wolcott (1933)
*Leptoglossus oppositus Say.	Chittenden (1902), Morrill (1910)
*Leptoglossus phyllopus Linn.	Drake (1920), Watson (1921), Adair (1932)

Pentatomidae	
Acrosternum hilaris Say.	Drake (1920), Schoene and Underhill (1933)
*Acrosternum pennsylvanicum DeG.	Drake (1920)
*Alcaeorrhynchus grandis Dall.	Watson (1921)
Euschistus variolarius P. de B.	Parish (1934)
*Nezara viridula Linn.	Watson (1918), Jones (1918), Watson (1921, 1929, 1930), Wilson (1923)
*Thyanta perditor Fabr.	Wilson (1923)

* Insects common in the South, but rare or absent in Connecticut.

Nezara viridula is the most important host of *T. pennipes* in the South, where the rate of parasitism may be as great as 93 percent (Wilson, 1923). In Connecticut, however, *Anasa armigera* and *A. tristis* are the hosts which can be considered important. When both *A. tristis* and *A. armigera* are present in equal numbers, the parasite exercises no apparent choice as to host, thus suggesting that availability is the chief factor in host selection. Of these two species, *A. tristis* is the more important host because *A. armigera* is available to the parasite for only a limited period of time. From hibernation, the latter appears in the squash field and starts oviposition from one to two weeks earlier than *A. tristis*—and before the appearance of *T. pennipes*. Soon after these appear, *A. armigera* diminishes in numbers, and within two or three weeks almost entirely disappears.

Packard (1875) was the first to give an account of the host-parasite relationship between the common squash bug and *T. pennipes*, although Cook (1889) was the first to identify the parasite. Subsequent authors to list *A. tristis* as a host species are too numerous to mention.

Morphology

Worthley (1924) has given a detailed account of the external morphology of the adult *T. pennipes* and discussed the genitalia. The internal reproductive organs have not been described previously.

There is nothing peculiar about the female reproductive system of this parasite as compared with other Tachinids of this type (Figure 9). Each ovary is composed of several ovarioles, the number varying among individuals, and even varying in the two ovaries of the same fly. Of 20 ovaries dissected, the smallest contained 11 ovarioles and the largest 16, with an average of 12.7 $\pm$ .29. In a fly freshly emerged, four oöcytes in varying stages of development can usually be observed in each ovariole. Since this would make a total of from 88 to 128 eggs, which is somewhat more limited in range than the number that may actually be deposited, presumably not all the eggs formed are to be found in the egg tube distal to the germarium at the time of emergence. About midway between the junction of the lateral oviducts and the external orifice, the ducts from the spermathecae and accessory glands open into the common oviduct by means of narrow ducts. The two accessory glands are sub-cylindrical in shape, although expanded near the ducts which lead to the common oviduct.

Worthley (1924) stated that the female genitalia are composed of a dorsal, a ventral, and two lateral valves borne by the ring-like eighth segment. These "valves" are not homologous with such structures in a

true ovipositor, but are rather terminal lobes borne by a sclerotized ring telescoped within the seventh segment (Figure 9). Although these structures have only a membranous connection with the seventh segment, it is very doubtful if they represent the eighth segment. Ventrally, the ring is a very narrow, heavily sclerotized, yoke-shaped band which can be seen through the seventh abdominal sternite and tergites. The broad, tongue-shaped ventral lobe arises from the median portion of this band. Laterally the band widens abruptly to form a broad, membranous inner portion, and a kidney-shaped, sclerotized outer portion which is visible externally as the lateral lobe. The small triangular dorsal lobe has a weak membranous connection with the inner border of each lateral lobe.

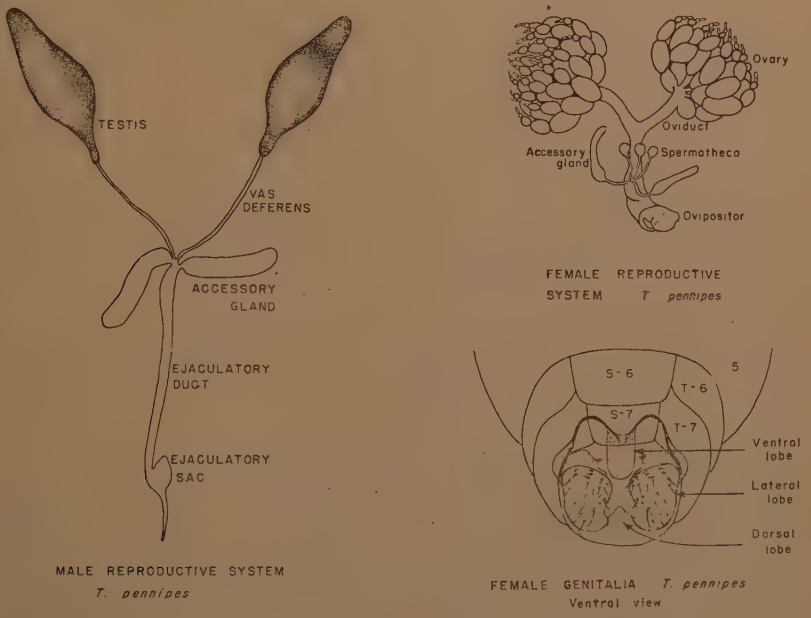

FIGURE 9. Reproductive systems of the male and female.

The male reproductive system is likewise in no way remarkable. The pyriform testes are deeply pigmented with a reddish-brown color. The vasa deferentia are narrow tubes which open into the proximal end of the ejaculatory duct just anterior to the openings of the accessory glands. The latter are sub-cylindrical in shape and communicate with the ejaculatory duct through a narrow neck. The ejaculatory duct is expanded somewhat proximally, and is terminated posteriorly by the cone-shaped ejaculatory sac. These structures are illustrated in Figure 9.

The male genitalia were illustrated and described in detail by Worthley (1924).

Developmental Stages

Egg

The egg of *Trichopoda pennipes*, which is deposited on the body surface of the host, has been well described

by Worthley (1924) as follows: "The eggs of *Trichopoda pennipes* vary in color from clear shining white to dirty gray, the coloration seeming not to depend on the age of the egg. The individual egg is ovate in outline, being slightly larger at one end. It is strongly convex, and is flattened on the side next to the body surface of the host. This flattened surface is covered by a colorless cement, by which the egg is affixed to the body of the host. The egg measures .56 mm. in length by .37 mm. in breadth, and its greatest height is .25 mm. The surface of the chorion appears smooth except under high magnification, when it is seen to be faintly reticulate in tiny hexagons. The chorion is comparatively thick and 'leathery', and remains rigid after hatching. The micropyle appears to be borne on a small papilla at the smaller end of the egg. Eggs which have hatched show a circular hole on the flattened side near the broader end. Since it is this flattened side which is pressed against the host, it is impossible to tell if an egg has hatched without first removing it from the body surface of the host."

Larvae

Worthley (1924) observed three larval instars, but believed that his dissections were insufficient in number to conclude definitely as to the number of larval instars. He thought that there might be four, as Townsend (1908) had indicated for other Tachinid parasites. Although Worthley did figure a second stage larva, the buccopharyngeal armatures of the second and third instar larvae, and the anal stigmata of the third instar, his account of the larval stages is quite incomplete.

As a matter of actual fact, Tachinid parasites characteristically pass through three larval instars, and *T. pennipes* is no exception to this. The stages can be distinguished readily by the buccopharyngeal skelton (cephaloskeleton) and the anal stigmata.

First Instar

The first instar larva is fusiform in shape, although somewhat truncated at the posterior extremity (Figure 10). It varies in length from 1.1 mm. to 2.4 mm., depending upon its stage of growth and upon the individual. The body is composed of the pseudocephalon and 11 externally visible body segments. The cuticle is colorless and transparent, so that the trachea and other internal structures are clearly visible. The integumental spines are numerous, but so extremely minute that high magnification and optimum illumination are necessary for their perception. The pseudocephalon is entirely devoid of these spines, but each of the 10 succeeding body segments is encircled anteriorly by a band of spines. The spines are uniform in size and shape, evenly distributed, and not clearly disposed in rows. On the terminal segment two spinous areas are present. A pre-anal band is conspicuous in the ventral region, but narrows abruptly towards the pleural region and is scarcely perceptible on the dorsal surface. The other group of spines is almost terminal in position and completely encircles an unarmed area surrounding the anal stigmata. Stigmatic hooks which are characteristic of larvae of this type and which supposedly serve to attach the larva to host tissue are lacking in this form.

The buccopharyngeal armature consists of a median tooth which bifurcates posteriorly. Each wing (representing a pharyngeal sclerite) consists of a broader dorsal portion and a narrower, sharply pointed, ventral portion.

The larva is metapneustic, with the posterior stigmata, which appear as merely split knobs, being terminal in position.

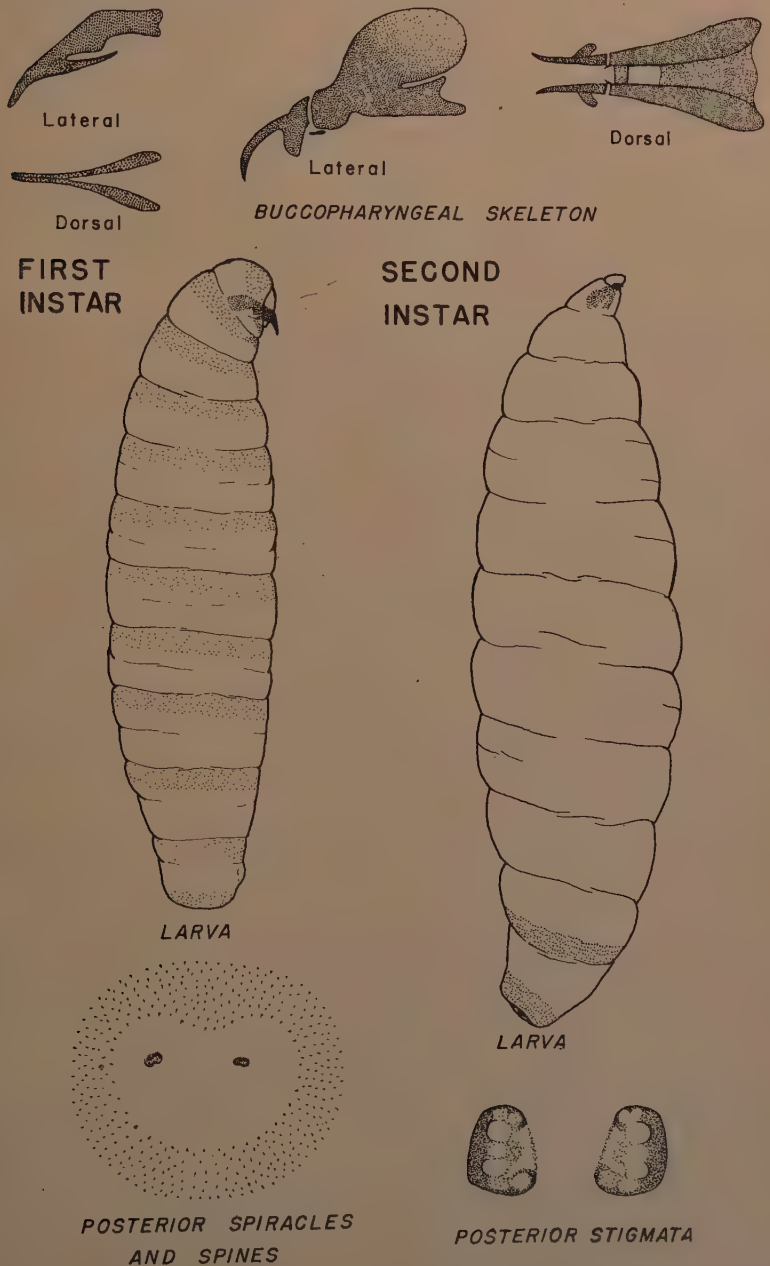

Figure 10. Diagram showing distinguishing characteristics of first and second instar larvae of *Trichopoda pennipes*.

Second Instar The body form of the second instar retains the same shape as that of the first (Figure 10). Length measurements indicate that considerable growth takes place during this instar and that the lower limit in size, 1.7 mm., overlaps with the upper extreme in the size of the first instar, while the upper limit, 6.7 mm., exceeds the length of the smallest third instar larva. The cuticle of this instar, as well as that of the third, is still colorless and transparent. The cuticular spines are somewhat more conspicuous than in the first instar, and their arrangement is more definite in that small groups of spines are arranged in a linear fashion, making irregular rows which completely encircle the body. There are but two groups of spines instead of the 12 found in the first instar. One of these forms a band around the posterior third of the penultimate segment. The other is in the terminal region of the last segment, similar to that of the first stage.

The cephaloskeleton undergoes considerable modification over that of the first instar. Instead of one median tooth, there is a pair of narrow, deeply curved, anterior hooks which widen abruptly posteriorly and which are well separated from the rest of the armature. The hypostomal and pharyngeal sclerites are still fused together but are much more conspicuous and more highly differentiated than in the first stage. Immediately below the anterior end of the hypostomal portion of the lateral plate and extending forward, is a very small rod-like sclerite which is paired and separated from the other sclerites. This is the infrahypostomal (parastomal) sclerite. It is probably represented in the first instar larva, although it is not distinguishable. The armature is not sclerotized uniformly, and the dorsal wing of the pharyngeal sclerite may be but weakly pigmented.

The posterior stigmata are considerably more conspicuous in this instar. Each spiracle consists of two short, horizontal slits, one above the other. The median border of each slit is weakly sclerotized and may show no pigmentation. The lateral border, on the other hand, is strongly sclerotized, with the result that the whole spiracle appears as an E-shaped sclerotized region.

Third Instar The body shape of this stage maggot cannot be considered fusiform, for while the anterior segments show a gradual tapering, the posterior half of the larva is quite cylindrical (Figure 11). When the maggot is contracted, both extremities have a rounded appearance. The terminal segment has a diameter conspicuously less than the preceding segments, and is the only one to possess the cuticular spines. The spines are somewhat more prominent, and the curved lineal groups are more distinctly separated than in the second instar. The range in size of this instar—from 4.6 mm. to 10.4 mm. in length—is unusually large and indicates wide variations among individuals as well as extensive growth. Furthermore, it is very remarkable that a parasite larva a centimeter in length can be supported by a host little more than 15 mm. long. Such a comparison is shown in Plate 1, F.

In shape, the buccopharyngeal skeleton of this stage does not differ much from that of the second. It is heavier, however, and the sclerites are more distinct. The anterior hooks are more robust than in the second instar and articulate posteriorly with the hypostomal sclerites. The latter are comparatively small and are joined ventrally by a narrow band.

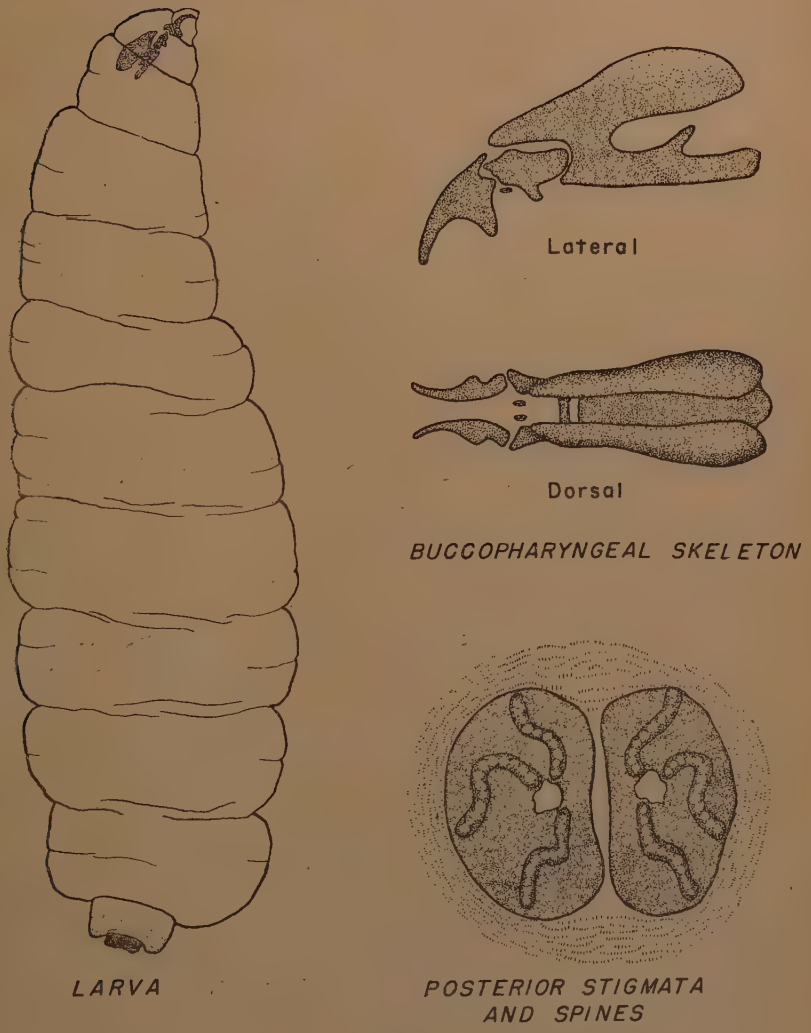

FIGURE 11. Diagram showing characteristics of third instar larva of *Trichopoda pennipes.*

In their lateral aspect the hypostomal sclerites are irregular in outline, but in their dorsal or ventral aspect the two sclerites with their connecting bar form an H-shaped configuration. The hypostomal sclerites articulate posteriorly with the prominent pharyngeal sclerites. In the posterior portion of each pharyngeal sclerite is a deep indentation which divides

the dorsal from the ventral wing. The ventral wings are not separated from each other, but are joined by a broad area which shows a reticulated sclerotization. Ventral and median to the anterior arms of the hypostomal sclerites are the two very small isolated sclerites—the infrahypostomal (parastomal) sclerites.

Well raised above the surface of the last segment are the posterior stigmata. Each of these consists of a well-defined button and three serpentine slits, the borders of which are raised slightly above the kidney-shaped peritreme. Immediately after the maggot has molted to this stage, the stigmata are straw-colored but soon darken and ultimately become a dense black. The extent of melanization is a good criterion for approximating the degree of development of the third instar larva.

The tracheal systems of the second and third instar larvae should be mentioned. Muscoid larvae characteristically possess an anterior spiracle which opens in the pleural region on the posterior border of the first body segment. The spiracular chamber arising therefrom communicates directly with the longitudinal tracheal trunk which terminates in the posterior spiracle. The anterior spiracles are customarily small in Tachinid larvae, but they are entirely lacking in *T. pennipes*, so that this larva may be said to be metapneustic in all three stages. Externally there is no evidence whatever of a spiracular opening, and internally the longitudinal tracheal tubes may be traced anteriorly to the prothoracic region where, instead of terminating in spiracular chambers, they break up into a number of very fine tracheoles which branch and rebranch to supply the integument. In the third instar larva a fine strand of tissue joins the anterior end of the tracheal trunk to the integument. It is possible that this represents a vestige of the spiracular chamber, but evidence for this is not convincing, and in the second instar maggot even the strand was not observed.

On the ventral side of the terminal segment, in all larvae, a slight protuberance represents the anal region. No lumen, however, is evident in the gut until just prior to the emergence of the parasite from the host.

Puparium

The puparium of *T. pennipes* has been figured by several authors, including Drake (1920), Greene (1921), and Worthley (1924). Characteristic of flies of this type, the pupal case is formed from the last larval skin. Accordingly, the posterior spiracles retain the shape of the third larval instar. The puparium is formed within a few hours after emergence from the host and is at first yellow in color, later darkening to a dull red. It varies considerably in size, ranging from 4.6 to 7.4 mm. in length and from 2.0 to 3.6 mm. in width, with a mean length of 6.3 mm. and a mean width of 3.0 mm.

Life History

Soon after emergence, the flies mate and the females seek the hosts upon the body surface of which the eggs are laid (Plate 1, E). Upon hatching from the egg, the larva drills through the body wall of the host and wanders freely in the body cavity. Just prior to the first molt, the larva attaches itself by its posterior extremity to a tracheal trunk of the host for purposes of respiration. The second and third instars are periods of rapid growth during which this tracheal attachment is retained. When growth is com-

pleted, the mature larva forces its way out through the posterior end of of the bug, falls to the ground, and pupates in the soil.

There are three generations each year, although the third generation may not be entirely complete. *T. pennipes* hibernates as a second instar larva within its host.

Worthley (1924) stated that the egg of *T. pennipes* hatches ir 30 hours (in Massachusetts), but in no case during the present study was hatching noted in less than three days. Drake (1920) observed that in Florida the eggs hatch in two or three days. Three and one-half days are required for the hatching of eggs maintained at a constant temperature of 78 degrees F. and a relative humidity of 62 percent. In the field also, the duration of the egg stage was usually three and one-half days, although cool weather might prolong this by as much as two days.

The time of development for the larval stages is 11 days when the parasitized hosts are kept at a constant temperature of 78 degrees F., relative humidity of 62 percent, and are supplied with fresh squash leaves daily. Two of these 11 days are spent in the first instar, four in the second, and five days in the third instar. Under field conditions, this time is somewhat prolonged. Insufficient data are at hand to indicate the duration of each larval instar in the field, but considering 85 parasitized squash bugs, death ensued in from 16 to 27 days after the deposition of the parasite egg, with a mean of 21.25 $\pm$ .06 days. Assuming the duration of the egg stage to be four days, and assuming that each bug lived approximately one day after the emergence of the larva, the duration of the three larval stages of *T. pennipes* approximates 16 days on the average, perhaps varying from 11 to 23 days.

The duration of the pupal stage in early spring, giving rise to the first generation of flies, was noted by Worthley (1924) to be one month. This appears to be correct. The pupae of the two later generations complete their development in much less time. In the field laboratory, with no control over temperature and humidity, flies were found to emerge in from 14 to 18 days after pupation of the larva, the average length of time being 16 $\pm$ .2 days. When the pupae are retained at a constant temperature of 78 degrees F. and a relative humidity of 62 percent, the pupal stage requires from 13 to 15 days, most of the flies emerging in 14 days.

Further details of this life cycle will be discussed in the succeeding chapter.

IV. HABITS, BIONOMICS, AND BEHAVIOR OF *T. PENNIPES* TOWARD HOST

Emergence of Adult Flies

The emergence of the flies occurs, within rather narrow limits, only at a certain time of day. From pupae kept under observation, flies emerged only in the morning, principally between the hours of 7:30 and 9:30 a.m.* It is likely that this holds true in the field as well, for there no flies were

* Drake (1920) also observed this in Florida and stated that the flies generally issued between eight and ten o'clock.

ever seen emerging except in the morning. No experiments were made in an effort to determine the cause of the regularity of this emergence, but it is probable that the rapidly rising temperatures characteristic of the early morning hours are a contributing factor.

Sex Ratio

Of 143 flies which emerged from pupae kept in the laboratory, 79 were females and 64 were males. The chi-square test indicates that the departure from a .5 sex ratio is insignificant.

Mating

At the time of emergence, the female flies possess fully developed eggs. Moreover, histological preparations of the testes of the males indicate complete maturity, in that no stages of spermatogenesis other than that of the mature sperm are to be found. These facts suggest a short preoviposition period, which is actually the case. Caged flies were observed to mate and oviposit within as short a time as two hours after emergence.

No evidence was obtained to indicate that a female was restricted in her mating to males of either her own or different ages, but mating seemed to be entirely at random. Polygamy is common, but in view of the relatively short life of the fly and its comparatively small reproductive capacity (see below), it is very doubtful if multiple mating is necessary for the fertilization of the full complement of eggs.

Longevity

Due to the fact that it is a far more active insect, *T. pennipes* is much less amenable to cage experiments than is *A. tristis*. Nevertheless, observation of caged material seems to be the only approach to the study of longevity of the fly. The cages employed were of 8-mesh hardware cloth, cylindrical in shape, seven inches in diameter, and 10 inches high. Only flies which had emerged from laboratory-reared pupae were considered. They were caged in pairs and were provided with water and sugar for food. Several squash bugs were kept in each cage for host material.

Longevity records thus obtained cannot be applied to field conditions, for in some cases it was evident that flies dissipated their energies in an effort to escape confinement. On the other hand, the flies were protected from wind, rain, and any possible predators. It appears, however, that *T. pennipes* is not a long lived insect. Of 53 flies considered, the average length of life was 6.06 $\pm$ .33 days, with a maximum of 18 days observed in one case. There was no significant difference in longevity between the sexes.

Host Finding

The pupae which give rise to the second and third generations of *T. pennipes* develop in the soil at the bases of the squash plants upon which the host insects had previously fed. Consequently the flies which issue from these pupae are in a geographical position favorable to their search for new host squash bugs. The overwintered larvae, on the other hand,

emerge from their hosts before the latter appear in the squash fields. Hence pupation and adult emergence of the first generation may occur at some distance from the available host population.*

The first appearance of *T. pennipes* in the field has not been observed to precede that of *A. tristis*, the dates of first appearance being as follows:

	A. tristis	*T. pennipes*
1936	May 25	June 2
1937	June 7	June 7
1938	June 2	June 8
1939	May 31	June 12

This does not necessarily imply, however, that the parasite is not attracted to the squash field until the bug is present.

In reaching the hosts, the parasites could be attracted either to the squash field, in which they seek their hosts, or to the host insects themselves. To which attraction *T. pennipes* responds was not satisfactorily determined. A few flies were tested in an olfactometer of the type used by McIndoo (1926) in which currents of air were drawn through two chambers, one containing squash bugs and one containing fresh squash leaves. The flies, exposed to these air currents, exhibited complete indifference. The fact that this indifference might be due to the confinement of the flies invalidates the conclusion that the olfactory sense plays no part in the finding of hosts by the parasite.

The phototropic responses of the squash bug and its parasite are in part antagonistic. The squash bug seems to be indifferent to light of comparatively low intensities, but negatively phototropic to light of high intensities. Thus bugs tend to avoid direct sunlight, but otherwise are apparently indifferent to light. Oviposition and feeding commonly take place on the undersides of leaves, where the light intensity may be only 200 foot-candles, while the upper side of the leaf, in full sunlight, may be exposed to 10,000 foot-candles.

The flies, on the other hand, respond positively to light. Not only do the flies congregate on the lighted side of a cage unequally illuminated, but they are able to detect the lightest side of a well illuminated cage. This definite light-response greatly facilitates handling of the flies. In catching them or transferring them from one container to another, it is only necessary to hold over the fly a glass or screen container pointed in the direction of the strongest light, and the fly obligingly enters the brighter container.

On any clear day, from June through September, these conspicuous flies may be seen hovering about squash plants in the field. Casual observation indicates that the males predominate in numbers. The reason for this is due not to an abnormal sex ratio, but to the fact that the females are actively seeking out the host insects and so are to be found amongst the foliage rather than flying at random above the plants, as do the males. The female parasites methodically fly around the bases of the plants,

* Inasmuch as the first squash bugs to reach the squash fields are not parasitized, it is assumed that bugs in the field constitute the only population available to the parasite.

occasionally coming to rest on a leaf, stem, or on the ground. Although no study adequate for statistical treatment was made relative to the position of the bug and its chance of being parasitized, bugs tied to various regions of the stem and both leaf surfaces were attacked. Even the propensity of the bugs to congregate under leaves which lie in direct contact with the ground does not insure against parasitism, for flies have been observed actually to crawl under such a leaf, there to parasitize the host. Parasites have even oviposited on bugs being held in the hand of the writer.

The efficiency of the searching-ability of the parasite will be considered in a later chapter.

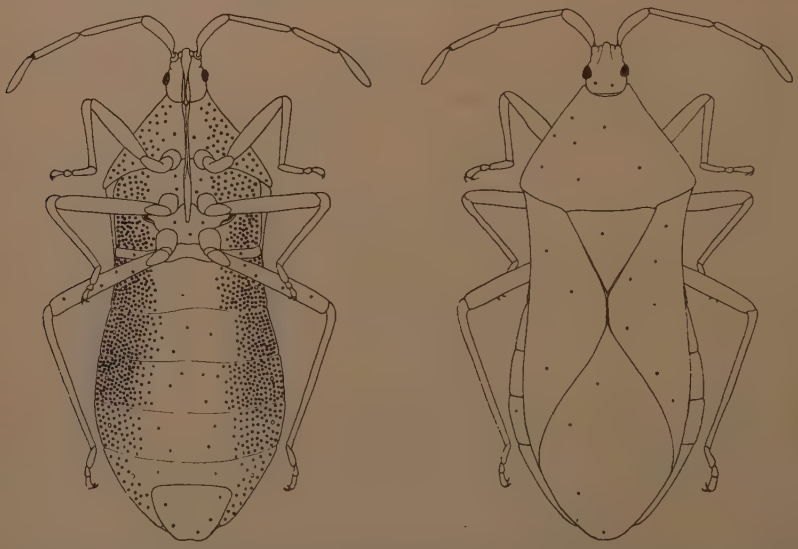

FIGURE 12. Diagram showing distribution of 1000 parasite eggs on *A. tristis*. Each dot represents one egg..

Oviposition

The act of oviposition takes place with great rapidity. The fly suddenly darts at the host and is away (seemingly), having scarcely touched the body surface as the egg was deposited. Caged parasites exhibit unusual behavior in that at times they may crawl indifferently over the body of the squash bug, which, in turn, is indifferent to the activities of the parasite. At these times, however, oviposition usually does not occur. This indifference indicates that the rapidity of oviposition is not essential because of any resistance on the part of the host. It would seem that this characteristic method of oviposition precludes the possibility of host-selection on the part of the parasite to avoid superparasitism. Observation of caged material indicated purely random oviposition, with some bugs receiving numerous eggs, and others receiving none at all.

The incidence of superparasitism in the field will be given more detailed treatment later, but here it might be stated that it is not uncommon to

find from 10 to 20 eggs on a squash bug, and as many as 48 eggs of *T. pennipes* have been observed on a single bug.

The eggs may be deposited on any part of the body surface of the bug, even occasionally on the legs and rarely on the antennae. The observed positions of 1000 parasite eggs deposited on squash bugs were recorded as follows:

DISTRIBUTION OF 1000 PARASITE EGGS ON *A. tristis*

Dorsal surface		Ventral surface	
Head	2	Head	0
Pronotum	7	Prothorax	60
Scutellum	1	Mesothorax	59
Clavus	2	Metathorax	99
Corium	10	Abdomen	
Membrane	5	II	2
Connexivum	2	III	157
		IV	195
		V	233
		VI	113
		VII	40
Legs	8	VIII-IX	5

The actual distribution of these 1000 eggs is shown diagrammatically in Figure 12 in which each dot represents a parasite egg. It is at once evident that the bulk of the eggs is deposited on the ventral surface of the bug's abdomen.

Reproductive Capacity

Oviposition records were obtained from female flies on which were made the longevity studies mentioned above. Oviposition is not normal under cage conditions as evidenced by the fact that of 33 females kept under observation, oviposition records were obtained from only 19 individuals. In only one instance did a female appear to lay her full complement of eggs, for the oviducts and ovaries of this individual were devoid of eggs after a total of 200 had been deposited. The oviposition data of the 19 insects from which eggs were obtained are summarized here:

OVIPOSITION OF *T. pennipes*

	Minimum number	Maximum number	Mean	P.E.M.
Eggs deposited on host insect	2	200	63.0 ±	9.2
Eggs in ovaries upon death of fly	0	93	43.4 ±	3.5
Total number of eggs	54	229	106.4 ±	7.1

Although the ovaries are fully developed at the time the fly issues from the pupae, and oviposition may occur within a very few hours after emergence, the full complement of eggs does not appear to be present at this time. Rather, some production of eggs continues during the life of the fly. In view of the one case mentioned above in which a full complement of 200 eggs was deposited, the number of eggs a female fly *can* produce probably does not far exceed this figure. In this study, the above

figure of 106.4 will be used as the average number of eggs the fly *does* produce. Moreover, due to the fact that oviposition in cages is not normal, this figure probably better represents the number of effective eggs than does the average of 63 eggs actually deposited on the host insects.

Egg Mortality

Egg mortality has not been determined because of the difficulties attending the detection of hatched eggs. It has been mentioned that it is not possible to observe whether or not the egg has hatched without first removing it from the surface of the host. Consequently, material gathered from the field could not be observed until it had been segregated for four days to allow sufficient time for all eggs to hatch. A recently hatched egg is easy to detect, for the circular opening through which the larva made its exit is clearly discernable. In an old egg, on the other hand, this region becomes discolored or otherwise obscured, whether the egg has hatched or not. The deposition of sterile eggs is infrequent in the field, but in cages it is not uncommon for the female flies to oviposit before mating.

Larval Penetration of Host

On the flattened side of a hatching egg, detached from a host insect, may be seen the black mouth hooks of the maggot rasping away the chorion to form the circular opening previously mentioned. In contact with the host, the maggot continues to bore through the body wall to gain entrance into the body cavity.

Worthley (1924) stated that the maggot, upon hatching, can penetrate the integument of the host regardless of the thickness of the cuticle at that point. This seems to hold true for the bugs which are parasitized the same year in which they mature. For example, of 95 parasitized bugs picked at random from the field in the fall of 1938, 93 bore parasites. On the other hand, of squash bugs which have overwintered, the cuticle of the entire thorax has become so heavily sclerotized that only a few of the maggots can penetrate this region. Similarly the wing surfaces of these bugs tend to exclude the parasites. Maggots which fail to penetrate the body wall in these regions perish, for they make no attempt to locate a more favorable point of entrance.

It is interesting to note that from an egg on the femur of the host, the hatching maggot has no difficulty in reaching the body cavity of the bug.

Habits of Larva Within Host

Within the host, the maggot at first takes up no definite locus, but may be found free in either the thoracic or abdominal cavity, supposedly satisfying its oxygen requirements by diffusion of gases through its integument in contact with the body fluid of the host. Growth during the first instar is relatively limited as compared with the two later stages.

Prior to the first molt the maggot makes its way to the thorax, if not already there, and attaches itself to a tracheal trunk of the host in order that its own tracheal system, through its posterior spiracles, will have direct access to an adequate oxygen supply. Just how this attachment is

made cannot be stated. As already mentioned, this maggot lacks the posterior hooks which in other forms are commonly supposed to serve this function. It may be that the cuticular spines are used, although they are exceedingly minute. This ability to attach itself does not seem to be confined to the first stage larva, as was shown experimentally. An etherized squash bug, containing a second instar parasite larva attached to the tracheal trunk of the left metathoracic spiracle, was opened in the left pleural region of the second and third abdominal segments.* The larva was then dislodged from its attachment and allowed to remain free in the body cavity of its host, after which the wound was closed and permitted to heal. At this time the tracheal funnel (described below) at the original attachment was well developed. After five days the bug was dissected. The maggot was found to be in a healthy condition and to have formed a new attachment, but this time to a tracheal branch leading from the right mesothoracic spiracle. A new tracheal funnel had not developed.

The choice of tracheae to which attachment is made is limited, with few exceptions, to the tracheae arising from the metathoracic spiracles. Out of 309 recorded cases, only five were attached elsewhere. These five were attached to tracheae leading from the mesothoracic spiracles. Of the 304 connected to the metathoracic tracheae, 146 were on the left side and 158 were on the right. The difference between these two figures is not significant, and it may be concluded that there is an equal chance of the larva occupying a position on one side or the other of its host.

No evidence of the failure of any maggot to reach the thorax has been observed, so that selective mortality does not seem to account for the constancy of the thoracic tracheal attachment.

There is no definite point on the metathoracic tracheae to which the maggot becomes attached. The attachment may be to the smaller tracheoles in the more distal region of a trachea, but more often than not it is to the proximal portion of a larger tracheal branch and quite near the spiracular opening itself. Here there is a sizeable unobstructed space in the angle formed by the metapleuron and the adjacent apodeme.

The irritation set up by the puncture of the trachea causes a reaction of the host tissue to form a funnel around the posterior extremity of the invading parasite. This funnel, brownish in color and heavily sclerotized, is comparable to that formed by other hosts attacked by Tachinids and is the "gaine de fixation secondaire" of Pantel (1910), who described it as an outgrowth of the tracheal wall and composed of a thick inner layer of chitin and an exceedingly thin outer layer of hypodermis.

That this funnel is an immunity reaction of the host is generally agreed, but its manner of formation may be open to question.* The tracheal funnel in many respects is very similar to the sheath formed around endoparasites of other insects. This encapsulation arises from an accumulation of phagocytes (Hollande, 1920) or of mesenchymal cells which assume the function of connective tissue cells (Lartschenko, 1932) to form a syncytium which later becomes heavily sclerotized and melanized.

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* For further details of the technique used, see appendix.

* Bess (1939) has written a recent review of the literature on host immunity reactions.

It is possible that a similar cellular accumulation takes part in the formation of the funnel in *A. tristis*.

Held in position by the funnel, the larva extends posteriorly into the abdominal cavity of the bug. The relative positions occupied by the second and third instar larvae are indicated in the accompanying diagram (Figure 13). Here again the extreme size of the mature larva as compared with its host is evident.

FIGURE 13. Diagram showing relative positions occupied by the second and third instar larvae in host.

The association between the larva and its enclosing funnel is not a very intimate one. This was well shown in the experiment earlier described, in which the connection was broken without injury to the larva, which then formed a new attachment. Moreover, too intimate an attachment would preclude the possibility of ecdysis, for the spiracles, as well as the skin, are shed at each molt. As it happens, the molted skin is sloughed off posteriorly and can often be found at the posterior end of the larva, between the latter and the base of the funnel.

Larval Mortality

It has been mentioned that larva from eggs deposited upon the thorax and wing surfaces of overwintered squash bugs perish by reason of their inability to penetrate the thick cuticle of the host. Inasmuch as 24.3 percent of the parasite eggs are deposited in these regions, it may be concluded that a similar proportion of larvae attacking overwintered bugs succumb.

Once within the body cavity of the host, a single maggot rarely dies; but in cases of superparasitism, many larvae may be destroyed.

The presence of supernumerary parasite eggs on the body surface of the squash bug has been mentioned. The incidence of superparasitism in the field may be judged by the fact that on 513 bugs, recorded during 1937 and 1938, a total of 1442 parasite eggs were deposited. Of these bugs, 55.5 percent bore more than one egg each. But in spite of such extensive superparasitism, the emergence of more than one maggot from a single host has never been observed by the writer nor reported by others. This implies the elimination of all but one larva.

Elimination of Superparasites

Considering the large size of a fully grown maggot, it is quite understandable why no more than one can mature in a single host. If the elimination is due to competition for space and food, the possibility should exist for two maggots in the second instar to be present—attached to the tracheae of both metathoracic spiracles. Although in two isolated cases two second instar larvae were found in a single host, in both cases the two larvae were side by side and attached to the same trachea. In all other cases observed, the destruction of all but one maggot occurred before the eliminated maggots molted from the first instar.

The question arises as to the nature of the elimination of the supernumerary larvae. Three possibilities might be considered: (1) direct competition for space, food, and other physiological needs of the parasite, resulting in the survival of the first one to gain the ascendancy; (2) secretion of toxic substances by the host or parasite; this implies an age threshold beyond which the larva is not affected, for otherwise all the parasites would be destroyed; (3) cannibalism, or the direct destruction of one parasite larva by another.

It is inconceivable that a squash bug can support a maggot as large as a third instar and no more than one first instar larva. Considering volume alone, it would require almost a thousand newly hatched larvae to occupy the space consumed by a single mature maggot. It is unreasonable, then, to consider that competition for space or food is responsible for the elimination of all but one larva.

The second possibility is approachable experimentally, and it is found that if there is any toxic substance secreted by the host or parasite it is not a general residual material, but must be very direct in its action. This was proved by the following experiment. Parasite flies were allowed to oviposit on a series of bugs. Five days later these same bugs were again subjected to parasitism. Three days after this the first parasite larvae, then in the second instar, were surgically removed.* The timing was such that the second parasites hatched and entered the body cavity of the host within a few hours after the removal of the first ones. In all successful cases (nine in number) the second parasites underwent normal development, being in no way affected by the earlier presence of another maggot.

The direct killing of one larva by another remains the most logical and probable mechanism of preventing superparasitism, although it has never

* See appendix.

been observed directly. It is common to find the shriveled bodies of excess parasites free in the body cavity of the host, but strangely enough, the integument, instead of giving evidence of violence, appears quite intact. Although severe injury of a mechanical nature clearly does not occur, a single puncture of the integument by the mouth hook of another maggot could pass superficial observation and yet initiate the destructive process. Such a puncture could be associated with the imbibition of the liquid contents and/or the secretion of proteolytic enzymes by the attacking maggot, but at present no conclusion can be stated with finality.

Emergence of Larva from Host

Emergence of the maggot occurs at the posterior end of the bug. Weed and Conradi (1902) noted emergence through the thoracic wall, but this has never been observed by the author, nor has it been reported by other writers. The point of emergence is not constant, but in the female it is quite usual between the subgenital plates of the seventh abdominal segment and the first pair of valvifers. In these cases there is a distortion of the parts, and the point of exit is obvious. Occasionally the maggot may emerge through the ovipositor itself, the valves of which fall back into place to disguise the external injury. The emergence from the male occurs through the intersegmental membranes of the genital segments, the point of exit being obscured by the telescoping of the ninth segment within the eight and seventh segments.

Internally, the former presence of a maggot is marked by several characteristics: Most convincing are the tracheal funnel in which the maggot was suspended, and the trail of fecal matter left by the maggot just before and during the process of emergence; the degeneration of the gonads; the more or less depleted condition of the fat body; and the collapse or destruction of the alimentary tract. The last condition seems undoubtedly to be caused mechanically by the extensive movements made by the maggot in forcing its way to the outside. The genitalia and associated structures suffer varying degrees of injury, depending upon the exact point of exit. If, for instance, emergence occurs through the valves of the ovipositor, the genital chamber is necessarily broken down.

It is quite probable that were it not for the mechanical injury caused by the emergence of the larva, a parasitized bug might complete its normal life span. As it is, death usually follows emergence within 24 hours, although it is not uncommon to find individual bugs surviving a longer period. No extensive records of survival were kept, but one individual was observed to live five and one-half days after the emergence of its parasite.

Pupation of Larvae

It has been mentioned that the larvae of the summer generations of *T. pennipes* pupate in the soil of the squash fields. But due to the fact that the larvae which give rise to the first generation of flies emerge from their hosts before the latter reach the squash plantings, it is not known exactly where pupation of these parasites occurs. While it is possible that the larva pupates at the site of the hibernation quarters, it more

likely does so after the host has become active in the field but before the search for food plants has begun.

Pupation normally takes place in the soil at a depth of about one inch. Undoubtedly soil texture and moisture relationships affect the depth to which larvae go, but no detailed studies were made in this regard. Mature larvae enclosed in glass vials will pupate normally, although no soil is provided.

The pupal mortality under natural conditions was not considered. The most important factors determining the mortality would seem to be soil texture and moisture relationships (with their modifications due to cultivation of the soil), predaceous organisms, and fungi. Under artificial conditions a pupal mortality of 27.1 percent was observed for a total of 70 pupae. These pupae were contained in cotton-stoppered glass tubes with a plaster of paris base. These tubes were kept in a field laboratory with no control over temperature and humidity. The latter was raised somewhat by the occasional addition of water to the plaster of paris. Even so, the pupae were probably drier than if they had been in the soil in the field.

Parasitism of Squash Bug Nymphs

Worthley (1924) noted that squash bug nymphs may become parasitized but die as a result unless the unhatched egg of the parasite is eliminated with the exuvia upon molting. Thus he stated that as many as 50 percent of the parasitized nymphs die before reaching maturity.

It is true that nymphs may become parasitized and that some parasitism is prevented by the casting off of the skin before the parasite egg hatches, but it is not true that parasitism necessarily results in the death of the nymph. Probably the 50 percent mortality recorded by Worthley was due to other causes, but because of the presence of the egg on the body surface of the nymph, the assumption was made that this was a causal rather than a coincidental relationship.

Very rarely a parasite egg is deposited on the body of a first instar squash bug nymph. It is extremely doubtful, however, that a maggot ever reaches the body cavity of this small host because the length of time required for the egg to hatch equals or exceeds the duration of the first instar of the nymph and so the unhatched egg is eliminated with the exuvia.

Whether or not a second instar nymph can support a parasite is not known, but since the appearance of a parasite egg on this stage is scarcely less rare than on a first instar it has little significance.

Similarly, no definite conclusion may be drawn in regard to the third instar, although, as would be expected, the incidence of egg deposition is more frequent than on the two preceding stages. Twenty-five third instar nymphs bearing parasite eggs were kept in a room maintained at a constant temperature of 78 degrees F. and a relative humidity of 62 percent. Such conditions were provided in the hope that better comparisons could be made in regard to time of development of these and older nymphs with their contained parasites. It became apparent, however, that food was a limiting factor in this case, resulting in retardation in the

development and high mortality of the nymphs. It was not possible to provide the nymphs with potted squash plants, so fresh squash leaves were given to them daily. Nevertheless, the caged nymphs did not feed normally. Of the 25, only six reached maturity and none of these bore any parasites. Such a result permits two interpretations. One is that the parasites caused the death of the nymphs with the exception of those six which avoided parasitism by eliminating the parasite egg along with the cast-off skin when molting. The other is that due to poor feeding conditions the natural mortality was very high and that it was purely by chance that the six survivors had avoided parasitism. The latter is the more likely, for in connection with another experiment it was found definitely that high mortality attends the rearing of nymphs under these conditions. An insufficient number of nymphs was used here to justify a statistical comparison, and the scarcity of parasitized third-instar nymphs prevented a repetition of the experiment.

Both the fourth and fifth instar nymphs can support the parasite. As many as 13 percent of the fourth instar nymphs and 22 percent of the fifth instar nymphs (present in the field at time of counting) have been observed to bear parasite eggs. That the parasite has little if any effect on the mortality of the nymph is shown in the following experiment.

One hundred fifth instar nymphs bearing parasite eggs and 100 such nymphs not bearing parasite eggs were kept under observation. Of the latter, the entire 100 matured, and almost as many, or 96, of the parasitized nymphs reached the adult stage. Although the death of the other four may possibly have been influenced by the parasite, such a figure lies well within the realm of chance and certainly does not justify the assumption made by Worthley. And of the 96 bugs subjected to parasitism, 74 actually bore maggots, the remaining 22 having avoided parasitism in one way or another.

In view of the unusual size of the full-grown parasite larva, it is difficult to understand this apparent lack of effect on the mortality of the smaller host if the course of development in the nymph is the same as that in the adult bug. That the course of development might not be the same was suggested by the following observations made in the summer of 1937: The duration of the entire larval life of the maggot in an adult bug (11 to 14 days) is little more than the duration of the fifth instar of the bug (7 to 10 days) and less than the time spent by the bug in the fourth and fifth instars together (14 to 19 days), and yet no parasites emerged from observed nymphs; of the few parasitized nymphs dissected, only first instar parasite larvae were found; in a group of fifth instar nymphs parasitized at the same time, those bugs which matured early yielded mature parasite larvae much sooner than those bugs which matured later.

These observations suggested a condition of suspended development of the first stage larva when in the body cavity of a nymph, and further observations made during the 1938 season proved such to be true. Thus, when in an adult host, a parasite larva spends only two days (at temperature of 78 degrees F., relative humidity 62 percent) in the first instar, but when in a nymph, the parasite remains in the first instar until the bug becomes an adult. This means that the duration of the first instar of the maggot may be extended by as much as ten-fold, as when a nymph is parasitized

early in its fourth instar. Within two days after the parasitized nymph reaches the imaginal stage, the parasite larva molts and continues its normal development. Thus the time spent as a first instar in a nymph may exceed the duration of the entire larval period in a mature host. Only two isolated exceptions were found to this delayed development, when second instar larvae were found in nymphs. Without knowing the total number of nymphs observed and dissected it is impossible to calculate the proportion of such cases, but these two certainly represent less than 1 percent of the parasitized nymphs.

Such a condition of suspended development is of physiological interest, for at no time is there any connection between the parasite and the tracheal system of the host. This implies that the respiration of the maggot is entirely through its integument in contact with the body fluid of the host. The tracheal attachment is made only after the final molt of the bug.

The cause of this phenomenon remains problematical. There do not seem to be any morphological differences between the older nymphs and the adult bugs sufficiently adequate to account for the behavior of the maggot. Physiological considerations seem to be the most promising. The increasing amount of knowledge relative to invertebrate hormones invites the thought that the phenomenon in question is regulated by such chemicals in the haemolymph. These could be in the form of growth-inhibiting substances in the nymph or growth-activating substances in the adult. The possibility of a molting hormone of the bug causing the molting of the maggot meets with two serious objections. In the first place, if such were the case, maggots in a fourth instar nymph would be expected to molt when the latter reached the fifth instar. And in the second place, maggots in adult bugs may enter the second instar in their usual short time despite the fact that their hosts may have had their final molt 10 months previously (as when overwintered bugs are parasitized). It is very doubtful that the haemolymph would retain a residual molting hormone for this length of time.

Transplantation experiments offer still more proof that the mechanism of molting in the host is not responsible for the molting of the parasite. These experiments, using the technique described in appendix, involved the transfer of a parasite larva from a nymph to an adult bug. In each of six successful transfers, the introduced maggot made its tracheal attachment and molted to the second instar within two days. The adults employed were of variable ages, including bugs which had recently matured as well as bugs which had overwintered, so no age factor need be considered. Due to the soft body of the nymph, the reciprocal experiment proved to be too difficult for real success. Although numerous attempts were made to transfer a first instar maggot from an adult to a nymph, the death of the latter usually occurred. In two cases only did nymphs survive so that dissection could be made after two days. In both nymphs the maggots had failed to molt. Although in this length of time the maggots certainly should have molted had they remained in adult hosts, other cases involving more extended periods are necessary for convincing proof.

Reciprocal blood transfusions between nymphs and adult squash bugs had no effect on the characteristic activities of the parasite larvae, but in the absence of quantitative control in the methods employed, negative results are not conclusive.

V. EFFECTS OF THE PARASITE LARVA UPON ITS HOST

Systemic Effects

It has been shown that for over two weeks a parasite larva can live within a squash bug, during which time it increases to a size almost equal to that of the body cavity of the host. But, in spite of this, the bug survives until after the parasite has emerged.

The general physiological effects on the host squash bug by the invading parasite have for the most part received only preliminary study. Consequently only a brief summary, without a critical discussion of methods and results will be given at this time.

No destruction of muscular or nervous tissue has been observed, and that functional activity of the musculature and nervous system is not impaired is evidenced by the fact that movements of parasitized bugs appear entirely normal.

No injury to the alimentary tract by parasite larvae prior to their emergence was observed, but when the maggot emerges from the host, the mechanical movement involved always results in the collapse and usually in the partial or complete destruction of the gut.

The food reserves in insects are largely incorporated in the fat body, which in the squash bug is composed of greenish lobulated masses. During the feeding season these fill practically all the space in the abdominal cavity not occupied by other organs. As would be expected, after hibernation or starvation the fat body is noticeably depleted. The parasite appears to have no significant effect on the amount of ether soluble substances, of which the fat body is the chief constituent. The following data represent the results obtained by the Analytical Chemistry Department of the Connecticut Agricultural Experiment Station in the Soxhlet extraction of about 70 male bugs in each of the four categories:

Ether Extractives of *A. tristis* in Percentage of Dry Weight

	Overwintered bugs	Bugs prior to hibernation
Normal	8.25%	26.18%
Parasitized	9.63%	24.37%

It appears that either the parasite larva is not strictly steatophagous or the host in some way compensates for any loss in fat that may occur.

Since the parasite larva retains its fecal matter within its body until the time of its emergence from the host, the anal excretion of a parasitized squash bug probably represents only its own metabolic wastes together with water and unassimilated food from the plant juices. Quantitative estimates of the excretion of a total of 37 bugs showed no statistically significant difference between the average amount of solid waste excreted by normal bugs and that of parasitized bugs. No qualitative studies were made on the excrement.

The coöperative respiration—necessitated by the tracheal attachment of the parasite—should reflect a modified respiratory intensity in the host-parasite complex. A microrespirometer of the Fenn (1935) type was used

in determining the respiratory intensity of four pairs of bugs. One bug in each pair was parasitized and kept under the same cultural conditions as its normal control. Determinations were made for each pair at (usually) two-day intervals. Figure 14 shows graphically the results of one pair of bugs brought into the laboratory from the field. The initial progressive decrease in respiratory intensity by both normal and parasitized bugs was probably occasioned by an acclimitization to laboratory conditions. No appreciable difference between the two bugs was evident until the parasite reached the third instar, when a definite increase in oxygen consumption

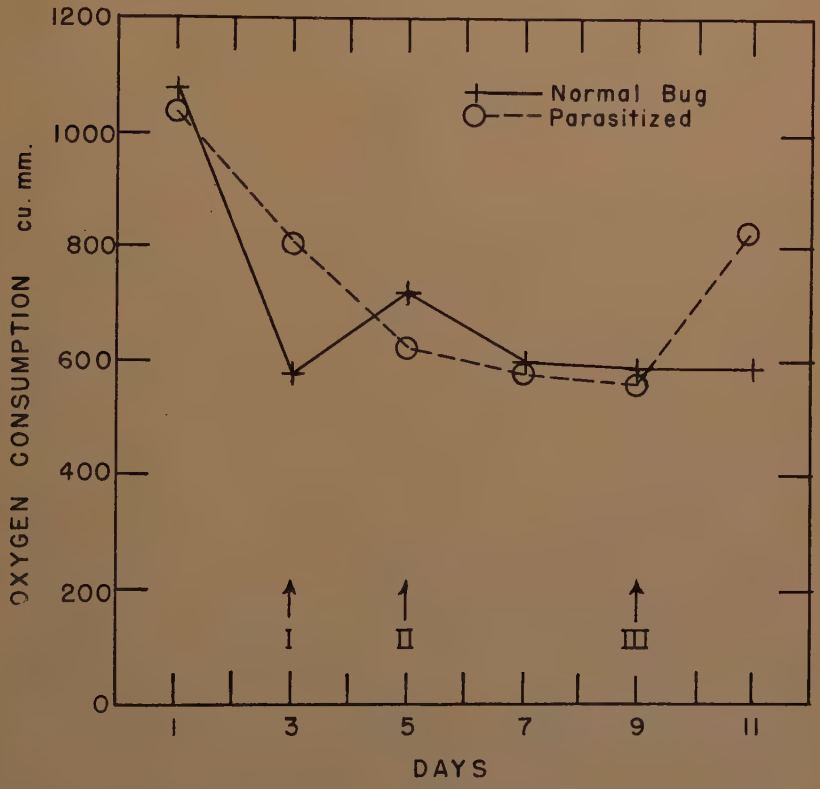

FIGURE 14. Chart showing oxygen consumption per hour by normal squash bug and parasitized squash bug. Numerals refer to time of molt of parasite.

occurred in the parasitized bug. A similar increase was noted in two other cases not illustrated here. Figure 15 illustrates data obtained from a normal bug and a bug containing a second instar larva in a hibernating condition. No significant departure from normal is observed in the oxygen consumption by the parasitized bug. This confirms the conclusion that, as tested, no increase in respiratory intensity is evident until the parasite reaches the third instar. It was found by testing the oxygen consumption of second instar maggots dissociated from their hosts that under the conditions of the experiment no measurable amount of oxygen

was consumed. Thus it is not surprising that the effect of the second instar maggot cannot be distinguished from the normal variations of the bug. The reason for the increased respiratory intensity of the bug when its parasite reaches the third instar, however, remains obscure. If the maggot is considered to be mere respiring tissue incorporated within the host, the relative level of oxygen consumption should remain the same, although the absolute consumption would increase because of the additional tissue. This, obviously, is contrary to the observed results, which indicated a marked rise in the relative oxygen consumption of a bug containing a third instar maggot. Determination of seven individual third instar larvae indicated an oxygen consumption of the same order of magnitude as that of the bugs, which makes an explanation of the increase in oxygen consumption still more difficult. One thing is apparent, however, and that is that the host suffers from no evident lack of oxygen.

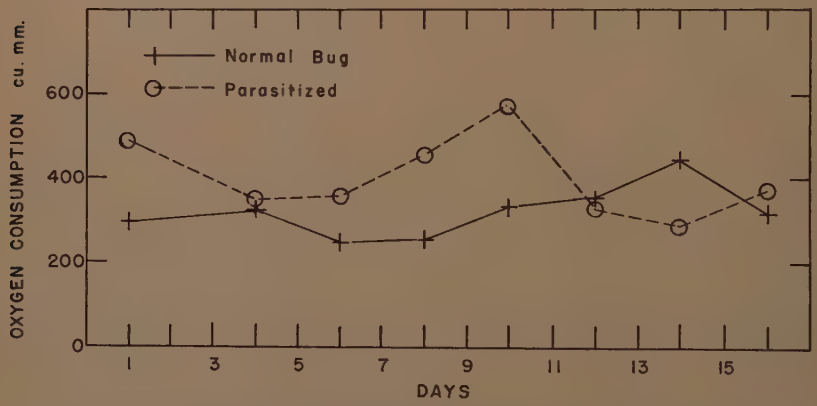

FIGURE 15. Chart showing oxygen consumption per hour by normal squash bug and bug containing hibernating parasite.

The work of Tauber and Yeager (1935) suggested that certain physiological conditions, such as the presence of a parasite, are correlated (perhaps causally) with an increase in the total cell number in the haemolymph of insects. They found that certain Orthoptera infected with bacterial and nematode parasites had a blood cell count higher than the range exhibited by normal insects of the same species.

These authors made a comparative study of the haemolymph cell counts of 33 species of insects, including *A. tristis*. Testing 16 specimens, using normal nymphs and adults of both sexes, they found the count to range from 228 to 4,913 per cubic millimeter of haemolymph, with an average of 1,637. This was the lowest average count found among the 33 species.

The writer made further counts, using essentially the same technique as Tauber and Yeager, to determine whether or not the presence of *T. pennipes* affected the total blood cell count. Only bugs which had passed through the winter were tested, since Muttkowski (1924) has pointed out that adult insects, particularly those which have passed through a hiber-

nating period, will possess more uniform blood constituents than insects in more active stages of development. Moreover, only males were used, since the work of Tauber and Yeager (loc. cit.) also indicated that there may be sexual differences, and in the females, egg production may modify the cell number.

A total of 100 counts was made, 25 in each category, listed below with the results obtained:

TOTAL NUMBER OF CELLS PER CUBIC MILLIMETER OF HAEMOLYMPH. *A. tristis*

	Min.	Max.	Mean		P.E.M.
Normal bugs	305	2811	1224	±	86
Bugs with 1st instar larvae	244	3299	1161	±	114
Bugs with 2nd instar larvae	555	5246	2117	±	156
Bugs with 3rd instar larvae	367	4033	1819	±	131

The figures show a more restricted range in the normal blood cell number than that indicated by Tauber and Yeager. This would be expected since the sampling was taken from a more uniform population. Although probably not significantly so, the mean shown here is lower than that observed by the other authors.

Except in the case of the second instar, the presence of the parasite appears to have no effect on the normal total cell number, for the departures from the normal mean are not of statistical significance. The presence of the second instar, however, is correlated with a significant increase over the normal count. In explanation, the only suggestion that can be made at present is the possibility that the formation of the tracheal funnel which occurs around the posterior end of the second instar parasite is correlated with, if not directly affected by, an increase in the cellular elements of the blood.

The only organs obviously affected early by the presence of the parasite are the gonads. In these a definite progressive atrophy is correlated with the development of the parasite larva.*

Parasitic castration among insects is not uncommon, but to the neglect of the physiological and ethological studies, interest in such castration has centered on the possible effects on secondary sexual characteristics. Wheeler (1910), Hanström (1937), and Wense (1938) have summarized the literature covering this subject, but the work of Pantel (1910, 1913) is of more direct relation to the case in point. Pantel distinguished between the direct effect on the gonad, in which a dipterous larva lives within the gonadial tissue, and the indirect effect, in which the larva resides outside of the gonad, exerting its influence through a systemic effect of a nutritional nature.

The onset of castration normally occurs one or two days prior to the time a second instar parasite molts to the third stage. The time varies somewhat as does the time of development of the larva, but almost invariably a squash bug bearing a well-developed second instar maggot gives evidence of gonadial atrophy. At this time, however, only one gonad

* A preliminary account of this phenomenon and its effect appeared in Jour. Econ. Ent. 33 (2): 269-272.

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shows degeneration—the gonad on the side of the bug to which the maggot is attached. Promptly after the larva reaches the third instar, the other gonad begins to atrophy.

That this phenomenon is a true degeneration and not merely a retardation of development is evidenced by two facts. First, the effect may occur after sexual activity has taken place; and second, histological studies show that affected gonads are in no way comparable to gonads in their early stages of development.

That the degeneration is due to the parasite and not to the natural termination of reproductive activity may be concluded from the correlation between the onset of castration and the development of the parasite, and also from the fact that the degeneration may be observed as early as June, whereas healthy bugs maintain reproductive activity until the time of their death or until the approach of unfavorable conditions in September.

Although atrophy is progressive and reaches its limit only after the third instar maggot is well developed, the initial symptoms appear rather suddenly.

A longitudinal section through a normal squash bug's testis shows the organ to be composed of seven lobes separated from each other by thin sheets of pigmented connective tissue similar to the external testicular envelope (peritoneal sheath). Ramifying throughout this connective tissue is an abundance of tracheoles. Within each lobe the progressive stages of spermatogenesis are clearly observed.* The characteristic appearance of the normal testis, in section, is illustrated by Plate 3, F.

The first symptom of the parasitic effect is a general shrinking of the entire testis. There is no evidence of any localized effect, although a curvature of its flattened plane, along its long axis, is not infrequent.

The castration is of the "necrotique" type described by Pantel (1913). The histological picture indicates that even at the early stage there is considerable destruction of tissue (Plate 3, G). This seems to be centered at first toward the posterior regions of the testis. The most obvious effect is the complete destruction of many of the spermatic cysts. In the early stages of atrophy, cysts towards the blind ends of the testicular lobes appear quite normal. And, in fact, isolated cysts in the affected areas appear normal, showing characteristic mitotic figures. Cysts in the process of breaking down show a loss of normal cytological details, though the wall be still intact (Plate 3, cf. I,J). The destruction of cysts is progressive, and ultimately no normal ones are to be found. The connective tissue in the affected regions seems far in excess of the normal amount. This, presumably, is due not to the formation of additional connective tissue, but to the loosening and spreading of the dense tissue forming the interlobular septa and the walls of the spermatic cysts. The nuclei of the connective tissue appear normal, but unusually prominent. Mature spermatozoa are clearly observable. These may be disposed in bundles, more or less normal in appearance, or they may be scattered individually and promiscuously throughout the tissue. A testis completely atrophied appears as

* The clarity of these stages, explained by the classic researches of Paulmier (1899) and the extending works of Foot and Strobel (1907), Morrill (1910), Wilson (1925), Gatenby (1931), Baumgartner (1933), and Henderson and Gardner (1935), has made this form a favorite object for cytological study.

a mass of connective tissue anteriorly, with a solid core of mature spermatozoa posteriorly (Plate 3, H).

The vasa deferentia and the seminal vesicles are not obviously affected.

The seven ovarioles comprising each ovary of the squash bug are of the acrotrophic type. During the season of reproductive activity, from three to five eggs in progressive stages of development may be seen in each ovariole, and sections show other oöcytes separating from the trophocytes in the germarium (Plate 3, C). Characteristic of acrotrophic ovarioles, plasmatic strands serve to conduct yolk-forming materials from the nurse cells in the germarium to the developing oöcytes. The follicle cells enclose each of the larger oöcytes as a wall one cell in thickness. The wall of the ovariole is exceedingly thin, and when distended by a well-developed egg, is scarcely perceptible in section. Sections of these larger eggs show the characteristic large, darkly staining, deutoplasmic granules.

As in the testes, affected ovaries at first show a shrunken condition (Plate 3, cf. A,B). Commonly the first point of injury is just posterior to the second or third well-formed egg in the egg tube (Plate 3, D). Posterior to this, more completely developed eggs may appear perfectly normal; and anterior to this, the germarium and oöcytes in earlier stages of development show no abnormalities. As injury becomes more pronounced, adjacent areas become atrophied. The eggs that may be present posterior to the original point of injury are deposited if development is sufficiently advanced, or they degenerate if not (Plate 3, E). The point of injury is characterized by a collapse and distortion of the egg tube. The wall becomes conspicuously thickened. It cannot be clearly determined whether this thickening is merely the ovariolar wall, released from its distention by partial destruction of the enclosed egg, or the wall in combination with the follicular cells surrounding the egg. The shape and number of cells involved in the thickening suggest the latter. Within the tubes, eggs may be seen in varying stages of disintegration. The yolk, so abundant in healthy eggs, disappears. Histological preparations suggest that the disappearance is due to resorption, but the possibility, to be discussed later, of an actual withdrawal of yolk by the parasite must be considered. When the degeneration is extreme, the ovarioles are no longer well separated from each other, but become agglutinated by fatty tissue and by adhesion of the extensive tracheation. In these cases, even the germarium becomes distorted, although the trophocytes and oöcytes remain distinguishable.

The posterior portions of the ovariole, pedicels, and the lateral oviducts give no evidence of any atrophy.

The question arises as to the nature of this castration. Pantel (1913) concluded that indirect parasitic castration is entirely a matter of nutrition. Specifically, the gonads suffer from an inadequate supply of nutrients, particularly of fatty substances. As will be shown, this does not explain satisfactorily the castration of *A. tristis*.

Two general possibilities may be considered: (1) that the parasitic castration is a systemic effect and (2) that it is a local effect arising from the immediate presence of the parasite larva. A priori, the systemic effect is highly questionable. In the first place, atrophy of one gonad begins

before that of the other by as much as two days or more. If the castration were due to a systemic effect, both gonads should degenerate simultaneously since they are symmetrically placed in the haemocoele. Moreover, the effect is not reversible in that once atrophy of the gonads sets in, recovery does not occur upon the removal of the causal agent. This was shown in three males and in one female. From each of these a second instar maggot was surgically removed at a time when only one gonad had begun to show degeneration. In the case of the males, dissection was made six days after the operation, and in each instance the one affected testis still showed marked degeneration, whereas the unaffected testis appeared perfectly normal. The effect was more clearly marked in the case of the female. This insect lived 33 days after the operation, and during this time deposited 76 eggs. Upon the death of this specimen, autopsy showed that the affected ovary was entirely functionless, and that the intact ovary presumably had produced all the eggs that had been deposited during the interval between the operation and death. These experiments show that the presence of the larva must continue in order for the second gonad to atrophy. In other words, there is no systemic effect of a residual nature.

The nature of the localized action which must be responsible is not at present known.

When the second instar larva occupies its normal position, its head region reaches the level of the gonad. The mouth hooks would thus be in a position to rasp away the tissues causing a purely mechanical injury, or secretions from the fore-gut or salivary glands might exert a cytolytic effect. But since the degeneration of the gonads is progressive and correlated with the development of the larva, a pressure effect must not be overlooked.

Observations have rendered questionable the possibility of mechanical injury alone causing the degeneration, for there is not the tearing of tissues that would be expected. The peritoneal sheath of the testis appears intact, and in the ovaries, each ovariole seems to be affected independently of the rest, with no general destruction of the egg tube. It is true that a small opening made by the parasite would pass unnoticed in gross observation and would not be convincing in histological preparations. Resorting to the operative technique, one testis in each of six bugs was pricked with a needle. A week later dissection disclosed none of the shrinkage or atrophy characteristic of the effect of the parasite. The original wound was evident, but it had not spread, nor was there evidence of any healing.

No technique was devised to test the effect of enzymes or other secretions of the parasite on the gonads in the living bug. On the other hand, gonads isolated from the body of the insect were treated with glycerine extracts of both the salivary glands and the fore-gut of the parasite larva. Neither of these extracts visibly affected the host tissue, whether accompanied by mechanical injury or not.

Methods of studying the possibility of a pressure effect have so far been unsatisfactory.

From this work no definite conclusions can be drawn as to the nature of the parasitic castration other than that it is due neither to a systemic effect nor to purely mechanical tearing of the tissues. The enzymatic

studies have not been exhausted, and the possibility of a pressure effect has not yet been eliminated convincingly. Perhaps the simplest explanation—but difficult to prove—is that through a very small aperture in the wall of the gonad, the parasite imbibes the liquid contents. The appearance of the affected ovaries lends itself to this view, but the more generalized effect in the testes suggests the assistance of proteolytic enzymes.

Without discussing the possible significance of glutathione, it might be mentioned that the nitroprusside test (Fink, 1927) for this substance is positive in atrophied ovaries as it is in normal ovaries.

Effect on Reproduction

The functional significance of the castration of *A. tristis* by larvae of *T. pennipes* remains to be considered.

In the male squash bug, the degeneration of the testes causes no reduction in reproductive activity. In the first place, the spermatozoa stored in the seminal vesicles are adequate for the fertilization of eggs. This was shown by mating a parasitically castrated male with a virgin female. All eggs deposited by the female as a result of this mating were fertile. In the second place, since a single copulation is sufficient to fertilize the full complement of eggs in the female (see page 610), and since mating begins in early spring before castration sets in, no loss of fertility occurs.

The case of the females is somewhat different, and while the castration effect may have little practical significance because death of the bug follows so closely upon the onset of castration, the phenomenon is of biological interest.

In one clear-cut case it was shown that the castration may take the form of an inhibition of gonadial development. A third instar maggot was surgically removed from a female squash bug which had just appeared in the field from hibernation (June 8, 1938). Other bugs taken at the same time showed incomplete development of the ovaries, and so it is assumed that the ovaries of this female, too, were underdeveloped. Although this bug lived a total of 36 days following the operation and mated frequently, no eggs whatever were deposited. Autopsy showed the ovaries to be in a state of complete atrophy.

Other operations, however, indicate that castration occurring later in the season is not in the nature of an inhibition of development, but an actual disruption of normal reproductive activity. Operations on gravid females are very difficult because of the contained eggs. This is particularly true when the third instar maggot is concerned, for the removal of so large an object imposes a great danger of mechanical injury to the internal organs. Although over 100 operations were attempted, the following tabulation represents an analysis of only those which can be considered in any degree successful.

In this series, cases 3, 8, and 9 were bugs taken in the field at the time of the operation, and so their previous oviposition record is not known. The other cases were bugs kept under observation from the time they appeared in the field after hibernation until their death.

OVIPOSITION OF *A. tristis* FROM WHICH PARASITE LARVAE WERE REMOVED

Case no.	Stage of parasite removed	Days survived after operation	Number of eggs deposited		
			Prior to parasitization	After parasitization: before operation	After operation
1	II	9	133	49	31
2	II	33	78	50	76
3	III	6	?	?	0
4	III	7	16	52	0
5	III	9	148	66	26
6	III	10	9	74	0
7	III	10	66	61	1
8	III	10	?	?	31
9	III	13	?	?	0

Although only two cases are presented from which a second instar larva was removed, it is evident that the degree of castration attending this period is not sufficient to inhibit completely the production of eggs. This is due to the unilateral nature of the early castration. It will be remembered that both gonads do not become affected until the maggot reaches the third instar. This unilateral effect was perfectly shown by case 2, which has been mentioned in another connection. When the maggot was removed from this bug, one ovary had degenerated. The other ovary, on the other hand, was intact and continued producing eggs for over a month after the operation.

At first glance the results observed in the other bugs appear variable, for in three cases eggs were deposited after the removal of a third instar maggot, while in four cases there were no eggs deposited. This oviposition at any rate, proves that the operation itself did not prevent the deposition of eggs. Moreover, this is quite in accord with the observation of the injury to the ovaries. It has been shown that the oviducts, pedicel, and even the well-developed eggs in the distal regions of the ovariole are not affected by the parasite. Accordingly it is quite possible for normal eggs to be resident in these structures at the time the ovary itself becomes non-functional. The eggs deposited after the operation represent such eggs as may have been fully formed at the time of castration. This conclusion is substantiated by the fact that autopsy of these bugs showed the ovaries to be entirely non-functional. It is for this reason, too, that parasitized bugs have been observed to oviposit up to within a short time before the emergence of their parasites.

From these studies it becomes evident that the oviposition of parasitized female bugs continues until castration sets in and the oviducts are void of eggs. The time required by the parasite to accomplish this can be estimated from oviposition records of 68 parasitized bugs observed in 1937 and 1938. The last egg mass was found to have been deposited on the average of 13.07 $\pm$ .07 days after the parasite egg was affixed to the host. It is now possible to express the average number of eggs a parasitized bug will deposit by the formula:

$$Y=4.7\ (X + 13.07)$$

in which Y equals the total number of eggs deposited, and X equals the time of parasitization, in days after the bug appears in the field after hibernation. (The figure 4.7 is the regression coefficient of the number of eggs deposited and the longevity of the bug, as discussed above.)

This formula was applied to 87 parasitized bugs from which oviposition records had been obtained. The theoretical average obtained was 110.7 ± 2.61 eggs per female squash bug. The average number actually obtained from these bugs was 105.7 ± 5.46. The small difference of 5 between these means is of no statistical significance, and the formula may be considered to yield a satisfactory estimate of the average number of eggs a parasitized bug deposits.

T. pennipes as a Parasite

A parasite is usually considered to be an individual which lives at the expense of its host without causing a lethal effect. This implies, in most cases, an adjustment on the part of the host to withstand the impairment caused by the invading organism. In entomological studies, the stylopization of Hymenoptera closely approximates this concept of parasitism, but in general, the so-called parasites among insects are actually internal predators or "parasitoids".

The observations presented in this chapter clearly indicate that while *T. pennipes* cannot be considered a true parasite, for ultimately the host is killed, the larval life of the parasite within the squash bug has little effect on the vital functions of the latter. It is true that the larva causes parasitic castration, but, however important the loss of reproductive function may be to the species, such loss is not vital to the individual insect. The single fact that a squash bug can live for as long as three months following the surgical removal of an almost fully grown maggot is proof of this.

To what extent the host adjusts itself physiologically to avoid the detrimental effects of the parasite remains problematical. That some adjustment does occur is suggested by the facts that respiration intensity increases as the maggot matures; that the cellular constituents of the haemolymph increase in number at the same time that a tracheal funnel is formed around the posterior end of the parasite; and that the amount of body fat in the host is not seriously depleted by the larva.

Such considerations point conclusively to the fact that death of the host is caused by conditions arising from the emergence of the larva. Chief of these is the mechanical destruction of host tissues by the body movements of the parasite, but the fecal discharge of the maggot may be a factor contributing to the early death of the squash bug after the departure of the larva.

VI. POPULATION STUDIES

Interrelationships of *A. tristis* and *T. pennipes*

THE synchronization of life histories of host and parasite may be outlined briefly as follows: There is only one generation each year of the squash bug, but there are three generations of its parasite. The third generation is not entirely complete, but is sufficient to be important. Winter is

spent by the hibernating squash bug in the adult stage, and by the parasite in the second larval stage within the host. In the early spring the parasite completes its development, emerges from the host, and pupates in the soil. The mature flies emerge and appear in the field at about the same time the squash bug makes its appearance. Both insects begin their oviposition. That of the squash bug continues until death or until the onset of unfavorable conditions in September. Only those bugs which have overwintered are parasitized by the first generation of flies, which begins to decline in July. The second generation, overlapping the first somewhat, appears during the latter part of July. At this time there are still some overwintered adult squash bugs, and these become heavily parasitized. But also at this time, the new generation of squash bugs is reaching the adult stage, and this, too, is attacked. Some of the parasite larvae of this generation, which hatch from eggs deposited during the latter part of August, remain in the second instar and will pass the winter in the body of the host. The majority, however, complete their development to bring about the third generation of flies which is most apparent early in September. By this time the original bugs have almost entirely disappeared, and only those of the new generation are available to the parasites. These bugs, with their contained parasites, constitute the hibernating population.

Inasmuch as the parasite hibernates in its larval stage within the host, the winter mortality of the latter must affect the numerical abundance of the parasites in the spring. There also exists the possibility of the parasite affecting the winter mortality of the host, although this is very doubtful if the results of present hibernation studies are indicative. The squash bugs caged in the fall of 1937 were parasitized to the extent of 25 percent. Of the survivors present in the spring of 1938, 30 percent were parasitized. And of the bugs caged in the fall of 1938, 22 percent were parasitized, whereas 25 percent of those alive the following February were parasitized. This implies, then, that whatever the winter mortality may be, the relative numbers of host and parasite are essentially the same in early spring as were present the preceding fall. Since a parasite larva emerges from the host before the latter appears in the field, each adult fly of the first generation represents an adult squash bug which did not live to reproduce.

These considerations have the paradoxical implication that, assuming no pupal mortality and a fall parasitism of over 50 percent, the population of the parasite in the spring would equal or exceed that of the host.* A fall parasitism of 50 percent or over is quite possible. In fact, observations made late in the fall of 1936 showed a parasitism of 75 percent, but since some of the adult bugs escaped parasitism by early departing for hibernation, the actual degree of parasitism was somewhat less.

Conceivably, with equal populations of parasite and host, a sex ratio of the parasite of .5, and 100 percent efficient host-finding, a reproductive potential of only 2 would be required for the complete parasitization of the host. An increase in the reproductive potential could compensate for unequal populations, and as a matter of fact, the power of increase of the parasite is far greater than that of the host. The number of offspring of

* Although not at any given time, for emergence continues over a period of about seven weeks. The bulk of the host population, on the other hand, may be present in the field at one time.

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a pair of squash bugs, assuming no mortality, would average 241, since there is only one generation each year, and this figure represents the average number of eggs deposited by a female protected from parasites and predators. On the other hand, the total offspring of a pair of *T. pennipes* per year would be 313,478 assuming an individual reproductive capacity of 106, a sex ratio of .5, 100 percent efficient parasitization, no mortality, and three complete generations each year. In other words, the power of increase of the parasite is over 1000 times that of the host. In spite of this fact, no occurrence of 100 percent parasitism has yet been observed, and it is doubtful if complete parasitization is possible for reasons given below.

Host Finding

It has already been noted that the flight of the female tachinid appears methodical and purposive as it seeks individuals of the host species for oviposition. However, Nicholson and Bailey (1935) have well pointed out that even if the individual search is systematic, as long as individuals search independently, the searching within a population is completely unorganized. And inasmuch as the parasite gives no evidence of discriminating between the sexes of the host nor between normal bugs and those already parasitized, oviposition on the adult squash bug is entirely at random. This very fact accounts, in part, for the failure of the parasite to exterminate the host. The reason for this may be illustrated as follows: Assuming a large host population and a single fertile female parasite capable of depositing 100 eggs, the probability is very high that 100 different host individuals will be parasitized. But if another similar parasite is introduced, the probability is not so high that an additional 100 different host individuals will be parasitized. Rather, some of the individuals will receive more than one egg. Consequently, an increase in the number of parasites will not result in a commensurate increase in parasitization, but an increasing amount of superparasitism will render many of the eggs ineffective. In this way, the degree of parasitism would tend to approach the 100 percent level asymptotically.

Thompson (1924), concerned with this problem of superparasitism, has presented a formula based upon host finding of a random nature.[1] This is:

$$y = N \quad (1 - e^{-x/N})$$

in which N equals total number of hosts, x equals number of parasite eggs distributed, and y equals number of hosts parasitized. Implications of this search-discovery relationship are also found in the work of Volterra (1928). For a wider application, Nicholson (1933) presented a curve to show the area traversed by an animal in relation to the area of discovery, and Nicholson and Bailey (1935) have given the formula, $u = u_1 e^{-s}$, in which u_1 is the number of objects originally present in a unit area, and u the number undiscovered after an area s has been traversed. The term x/N in the Thompson formula can be considered as representing the same factor as s in the formula of Nicholson and Bailey, in which case the two formulae are found to be indentical.

[1] Walker (1937) has made a mathematical analysis of superparasitism of *Cephus pygmaeus* Linn. *by Collyria calcitrator* Grav., in which host finding is not at random.

Thompson's (1939) criticism of Nicholson and Bailey's (loc. cit.) concept of "random search" does not modify the above except perhaps in the definition of terms.

This expression cannot be applied strictly to the available data on superparasitism of the squash bug, which might indicate that host finding by *T. pennipes* in the first generation is not at random. Nevertheless, if data from 38 observations made periodically during the course of three seasons are considered, parasitism of the host appears to be a logarithmic function of the parasite population. This is illustrated in Figure 16, in which the logarithm of the percentage of bugs escaping parasitism is plotted against the number of parasite eggs deposited per 50 bugs. The correlation coefficient of these data is .944, which is highly significant, being well beyond the 1 percent level. The formula of the regression line, derived by the method of least squares, may be expressed in the exponential form of

$$y = 89.736\ e^{-.01027\,x}$$

in which y equals the number of squash bugs escaping parasitism, and x equals the number of parasite eggs per 50 bugs.*

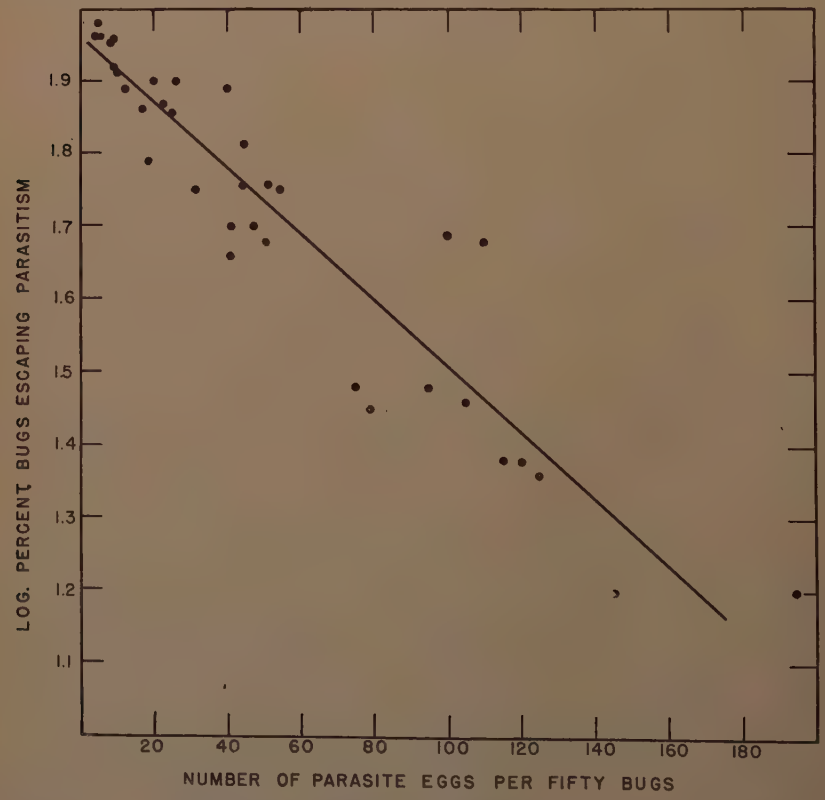

FIGURE 16. Chart showing logarithm of percentage of bugs escaping parasitism in relation to the available number of parasite eggs.

* The exponential formula assumes a large host population. But for the first generation of parasites, to which this formula alone applies, a large host population is usually available.

The wasted effort of the fly by superparasitism becomes even more strikingly apparent if specific examples are given. On July 10, 1937, when parasitism was at its peak, 42 out of 50 bugs bore a total of 194 eggs. Thus eight hosts, or 16 percent, were not parasitized in spite of the fact that the parasitized bugs bore on the average almost five eggs each. Again, on June 27, 1938, of 140 host insects, 116 bore a total of 405 eggs. Thus 17 percent of the bugs were not parasitized, although the number of eggs available was 2.9 times that necessary for complete parasitization if the eggs were properly distributed.

The oviposition by the flies of the second and third generations is less random due to the fact that the immature stages of the squash bug constitute a part of the host population. But parasitization of the nymphs is not proportionate to their share of the population, for the parasite shows discrimination in favor of the older nymphs and adult insects. This was observed with caged material, where nymphs were parasitized but rarely, and is also borne out by field observations. Data below show the relative number of individuals which bore eggs of *T. pennipes* at different times during the late summer of 1937. Although at that time of the season the nymphal population exceeded the adult population, the proportion of fifth instar nymphs that were parasitized was less (or no greater) than that of adult bugs. Fourth instar nymphs were even less heavily parasitized, and the number of parasite eggs on smaller nymphs was insignificant.

PERCENTAGE OF BUGS BEARING PARASITE EGGS

1937		Fourth instar nymphs	Fifth instar nymphs	Adults
August	7	12%	16%	20%
	14	9	22	22
	21	2	8	8
	28	1	2	8
September	4	2	12	18

The presence of eggs on the body surface of a squash bug should not be considered the only criterion of parasitism for bugs of the new generation. This criterion is fairly reliable for bugs which have overwintered, for although eggs deposited on the very thickly sclerotized regions of the thorax and wings may fail to yield effective parasites, there are often other eggs present which may compensate for these. Even so, of 130 squash bugs of the overwintered generation, all of which bore eggs of *T. pennipes*, 18.5 percent failed to harbor parasite larvae. Thus the extent of parasitism, estimated on the basis of the presence of parasite eggs, is too high. On the other hand, such an estimate is too low when this criterion is used for bugs of the new generation. In the first place, the body wall of the bug has not become sufficiently sclerotized to restrict the entrance of the larva, so it is very unusual for a parasite egg on such a bug to fail to give rise to an effective larva. In the second place, bugs of this generation may have become parasitized in their nymphal stages, in which case the parasite egg-shells would have been cast off with the exuviae when the nymphs molted. Consequently the extent of parasitism of these hosts can be determined only on the basis of dissection.

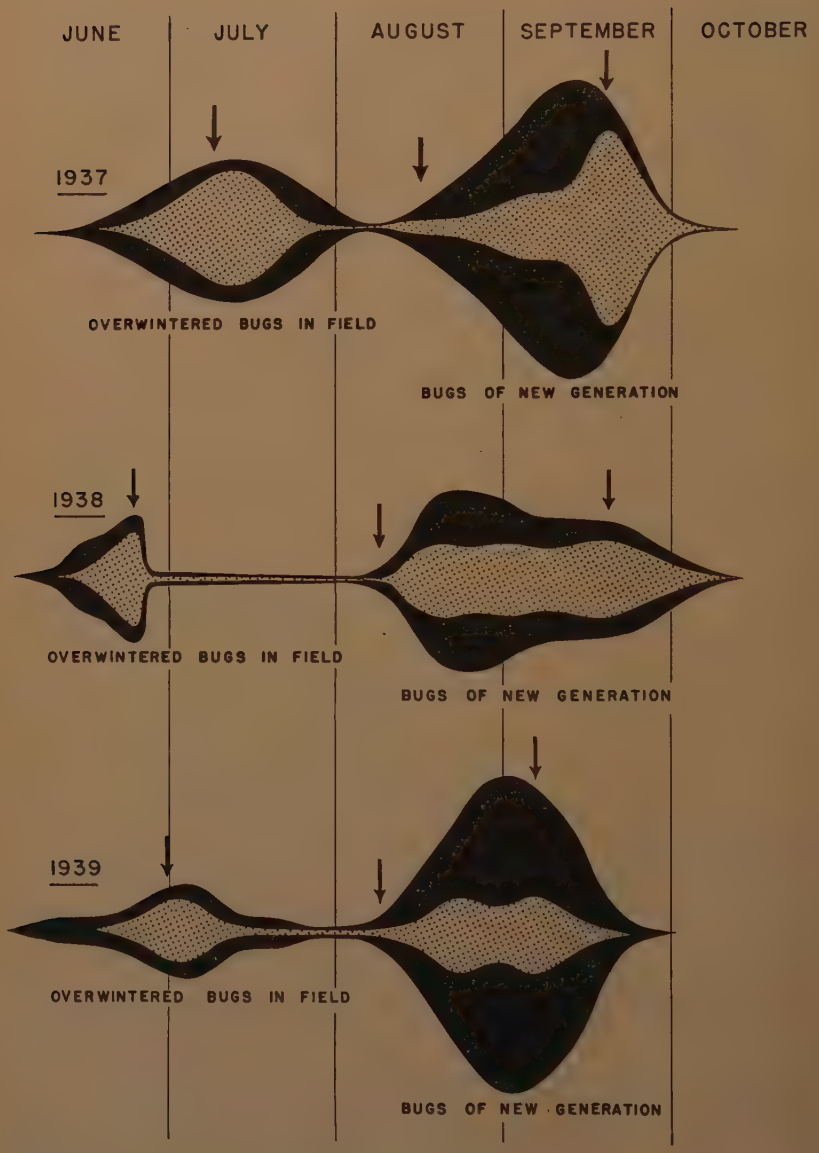

FIGURE 17. Chart showing seasonal population of squash bug. Width of each band represents total number of bugs present at given time. Stippled areas represent parasitized portion of population. Arrows indicate time of maximum abundance of adult parasites.

Synchronization of Life Histories

It is now possible to examine more closely the synchronization of the life histories of the host and parasite. The accompanying chart (Figure 17) indicating the trends of the populations for 1937, 1938, and 1939, is drawn as near to scale as the available data permits. Some of the numerical data will receive more detailed treatment below, but general trends become obvious when this chart is analyzed.

In this chart are represented the adult host populations with their larval parasites. Only the populations as they appear in the field are considered, for the hibernating populations are of little concern in the present analysis. The width of any part of each band represents the relative number of individuals present at that time. The adult host population for each season represents the end of one generation of bugs and the beginning of another. That portion of the band labeled "overwintered bugs" illustrates the population after emergence from hibernation quarters. The offspring of this generation is represented by the area in the chart labeled "bugs of the new generation". The stippled areas within each solid band illustrate the population of the parasite larvae which live within the body cavities of the hosts. It also may be considered that the solid portions of each band represent the population of non-parasitized bugs, whereas the stippled areas within them show the relative numbers of parasitized bugs. The arrows above each band indicate the time of maximum abundance of the adult parasites. Data for the larval populations presented in the chart are based upon periodic counts of parasitized bugs in the field and a consideration of the host population present at the time of counting.

The population density for the squash bug followed essentially the same trends for 1937 and 1939. The bugs began to appear in the field early in June and gradually increased to a maximum the early part of July. The 1939 peak occurred about a week earlier than the 1937 peak. During July the bugs of this generation rapidly died off, and by the latter part of August few were to be found. But during the first week in August the bugs of the new generation—offspring of the overwintered bugs—began to reach maturity. The number of nymphs reaching the adult stage increased rapidly during the month of August. In early September, however, the bugs departed from the squash field for hibernation quarters in increasing numbers. Although there was a continued increase in the total population with the addition of the newly maturing squash bugs, the rate of departure (added to the natural mortality) exceeded this increase to result in a marked depletion in the population resident in the squash field. Thus the field population was completely depleted in October.

In 1938, the early field population of adult *A. tristis* increased at a more rapid rate than in the other two years, and what appeared to be the onset of a heavy infestation was terminated by the heavy rainfall of June 26 to 28, when the bulk of the population disappeared. Although many eggs had been deposited already, and there were a few residual bugs to continue oviposition, a diminished population was observed throughout the season. Presumably due to this one unusually heavy rain storm, which was general throughout New England, the squash bug was not present in its usual

numbers throughout the State. No very heavy infestations were observed and none were reported to the Experiment Station.

The abrupt loss of the reproducing squash bugs caused the nymphal population to remain at a more or less uniform level after the time of hatching of all eggs laid prior to June 26. For this reason, the adult bugs of the new generation reached a peak of abundance when the nymphs from these eggs matured. This occurred considerably before the expected peak as judged by the 1937 and 1939 data. The field population of squash bugs then began to decline due first to mortality and then to departure for hibernation quarters.

Considering now the larval population of the parasite, the trend of the first generation closely parallels that of the host. This is to be expected, for the adult parasite appears in the field shortly after the bug itself appears, and increases to a maximum coinciding in time with the peak abundance of the host. In spite of this correlation, it must be remembered that the parasites may not be effective in inhibiting oviposition for about two weeks. Accordingly a large population of bugs is capable of extensive reproduction. Undoubtedly early parasitism effects a lowering of the peak of the host population, and later parasitism greatly accelerates the rate of decline of the host population, but the fact remains that the first generation of flies exerts its influence too late to be considered highly efficient as a control against the reproducing squash bugs.

There is some overlap between the flights of the first and second generations of flies, but during the third week in July flies are usually relatively few in number. The second generation of the parasite is in flight during the month of August, but in each of the years under consideration, the maximum population of the fly occurred during the first two weeks in August. Of particular importance is the fact that at that time, the population of the overwintered bugs had almost entirely disappeared. Moreover, relatively few bugs of the new generation had reached the adult stage, so that by far the bulk of the host population was comprised of nymphs of all instars. Since only nymphs of the fourth and fifth instars become parasitized to any significant degree, and even these do so to a limited extent, the number of hosts effectively parasitized was not commensurate with the population of adult *T. pennipes*. Although the host population was on the increase during August, the larval population lagged behind because of the declining population of *T. pennipes*' flies.

Also in each of the three years, there was considerable overlapping of the second and third generation of flies, and it was not possible to separate the two on the basis of field observations. The flies of the third generation, augmenting the effect of the declining second generation, caused an increase in the larval population which became most marked when the fly population reached its maximum in September. But in each year, the peak of the parasite population failed to coincide with the peak of the host population, but came after many of the bugs had left the squash fields.

It has been mentioned before that some of the larval offspring of the second generation of *T. pennipes* remain in the second instar for hibernation, thus rendering the third generation of flies incomplete. It is not

known to what degree this is done, but cursory evidence indicates that only a small proportion of the larvae fail to produce a third generation of flies. On the other hand, it is possible for larvae to complete their development too late in the season to produce effective flies. In 1938 flies were observed to emerge after all the bugs had departed for hibernation.

Because of its high potential power of increase, *T. pennipes* is theoretically capable of exterminating its host. But actually, the squash bug is ever present in abundance. It is apparent from the above discussion that the reason for this lies in the inefficiency of the parasite. This inefficiency may be attributed to the two factors summarized as follows:

1. The parasitism of the host is a logarithmic function of the oviposition of the parasite because of the fact that much of the effort of the latter is wasted through superparasitism.
2. The life history of the parasite does not sufficiently synchronize with that of the host for greatest efficiency.
   a. Since the parasite of the first generation affects overwintered adult hosts, and since the latter begin oviposition before the larval development of the parasite can be completed, many squash bug eggs are deposited. Thus the first generation of the parasite is effective too late to inhibit a large proportion of the host reproduction.
   b. The second generation of flies reaches maturity too late to parasitize effectively the overwintered bug population, and too early to exterminate the new generation of bugs.
   c. The third generation, reaching its peak abundance after many host individuals have left the squash fields for hibernation, likewise is not capable of utilizing its full reproductive powers.

These facts suggest that, if they synchronized more closely with the host life history, two generations of the parasite might be a more effective control against the squash bug than the three generations which now pertain.

Although the above considerations hold true if interest centers around the biological control of the host insect, the effectiveness of the parasite must be considered from another point of view. And that is, the economy of the parasite species itself. It is clearly obvious that if, in the absence of other hosts, the squash bug were exterminated, the parasite likewise would be destroyed.

It has been elucidated by Nicholson (1933) that animal populations bear a relation to the environmental conditions to which they are subject, with the implication that the populations must be in a state of balance with their environments. Moreover, for each species there is a particular density referred to as the "steady density", at which this balance exists. About this steady density occur oscillations, caused by the interaction of various environmental factors. In the maintenance of this steady density there must be a controlling factor which acts more severely against an average individual when the density of animals is high, and less severely when the density is low. The factor best satisfying this need is that of competition, of which the host-parasite relationship is a special form.

Apropos of the interaction of a specific parasite and a specific host, Nicholson (loc. cit. p. 149) noted that "The steady state exists when there is a sufficient density of parasites to destroy exactly the surplus of hosts

produced, and when there is a sufficient density of hosts exactly to maintain the density of parasites necessary to destroy this surplus. Thus there is a mutual adjustment of the steady densities of interacting hosts and parasites; the parasites destroy the surplus hosts, and the hosts maintain the parasites while preventing any surplus of parasites from being produced."*

The significance of this as applied to *T. pennipes* as a species, is the paradoxical fact that if it were more efficient it would be less successful. This is suggested by Nicholson and Bailey (1935), for since in the steady state a parasite destroys exactly the surplus of hosts produced, and since an efficient parasite would maintain its host at a low level, only a low density of an efficient parasite could be supported by its host.

The Parasitic Factor in the Control of *A. tristis*

It has been shown that *T. pennipes* does not maintain *A. tristis* at a low population density because of its inefficiency in host-finding and because of the incomplete synchronization of life histories. There now remains the consideration as to what extent the parasite does hold its host in check.

Interest in controlling the numbers of the squash bug centers around the reduction in damage caused by this insect to cucurbitaceous plants. In this regard, the overwintered bugs are of little direct concern, for their numbers constitute no serious menace. But since these bugs comprise the reproducing population, the value of the first generation of parasites lies exclusively in its ability to reduce the reproductive powers of the host squash bug. The nymphs and mature bugs—progeny of the overwintered bugs—constitute the principal feeding population. These insects are parasitized by the second and third generations of *T. pennipes*. But since a larval parasite in a nymph is not effective until the latter matures, flies of these generations are considered to be strictly parasites of the adult host. Such parasitized bugs, it will be remembered, never live to reproduce.

For purposes of this discussion, *T. pennipes* is considered to act upon a given generation of *A. tristis* first, as an oviposition suppressor, and second, as a parasite of the mature insect.

In the analyses to follow, data for three years are considered. Unfortunately, complete data are not available for each year, and some assumptions are necessarily made. Although future work may modify the values presented, the estimates should be indicative of the magnitude of the effect of *T. pennipes* in reducing the numerical abundance of the feeding population of *A. tristis*.

Effect of the First Generation of *T. pennipes*

The pertinent field data include estimates of the population density and the extent of parasitism. These are given for the three years on the basis of 30 hills of summer squash. For 1938 and 1939 the population density was determined by trapping and counting the bugs daily, but calculating on a weekly basis. For 1937, the population density was

* This extinction of hosts is a predatory function, not a classically defined parasitism, in which the host species is adapted to the parasite species.

estimated from oviposition records and the average oviposition rate of 4.7 eggs per female. The percentage of bugs parasitized was based on counts made at weekly intervals.

NUMBER OF OVERWINTERED BUGS PRESENT ON 30 HILLS OF SUMMER SQUASH AND EXTENT OF PARASITISM

1937			1938			1939		
	No. of bugs	Percent parasitized		No. of bugs	Percent parasitized		No. of bugs	Percent parasitized
June 19	3	28%	June 13	79	18%	June 20	24	13%
26	24	44	20	82	44	27	53	60
July 3	21	76	27	140	83	July 4	93	77
10	42	84	July 4	10	50	11	96	49
17	126	76	11	8	42	18	61	37
24	39	72	18	5	53	25	34	17
31	18	44				Aug. 1	19	35
Aug. 7	9	21				8	13	45
14	3					15	7	23
						22	3	4
						29	1	7

Proper use of these data can be made only if a number of qualifications and assumptions are considered. These are listed as follows:

No squash bugs become parasitized prior to the time they appear in the squash fields.

As the bugs emerge from hibernation and appear in the fields, there is an excess of immigrants over emigrants until the maximum field population is reached; after that immigration equals emigration to effect a stable population (see page 619), disregarding the factor of mortality.

The natural mortality, exclusive of that caused by predation and parasitism, is assumed to have followed the 1938 trend for caged material (see page 620).

The average daily oviposition rate is considered to be 4.7 eggs per female (see page 616).

The parasite shows no discrimination between the sexes of the host.

Of the apparently parasitized bugs, 18.5 percent fail to bear effective parasites (see page 638).

It is possible for a parasitized bug to succumb from natural causes before the effect of the parasite is realized.

The parasite requires two weeks to effect parasitic castration (see page 654).

The parasite requires three weeks to destroy its host (see page 633).

With this information in mind, it is possible to calculate the data in two ways: (1) To derive from the observed population a theoretical population subject only to natural mortality. The difference between the expected oviposition from such a population and the oviposition

actually obtained would thus be an estimate of the amount of oviposition suppressed by the parasite. This estimate would be a maximum figure, for it assumes that all suppression of oviposition not due to natural mortality is attributable to the parasite, thus neglecting any predatory effect or the possible underestimate of natural mortality. (2) To calculate the actual number of bugs effectively parasitized and the loss of reproductive activity due to the parasite. This gives a minimum figure, and probably one more nearly correct.

The results obtained are as follows:

EXTENT OF SUPPRESSED OVIPOSITION

	Minimum	Maximum
1937	28%	42%
1938	3	5
1939	11%	

The low figure obtained for 1938 can be traced directly to the heavy rain storm previously mentioned, which by destroying so many parasitized bugs prevented the full effect of this parasite generation from being realized.

Effect of the Second and Third Generations of *T. pennipes*

The effect of the second and third generations of the parasite can be calculated from the following estimates of bug populations and degrees of parasitism:

NUMBER OF BUGS OF NEW GENERATION PRESENT ON 30 HILLS OF SUMMER SQUASH AND EXTENT OF PARASITISM

1937			1938			1939		
	No. of bugs	Percent parasitized		No. of bugs	Percent parasitized		No. of bugs	Percent parasitized
July 31	11		Aug. 4	11		Aug. 8	33	45%
Aug. 7	81	20%	11	133		15	94	23
14	66	34	19	30	55%	22	181	30
21	139	22	26	37	25	29	270	17
28	419	26	Sept. 2	22	33	Sept. 5	180	30
Sept. 4	131	22	9	33	33	12	140	27
11	55	28	16	33	54	19	41	26
						26	7	

For 1937 and 1938 the populations of bugs were estimated from oviposition records, considering the egg to adult life cycle to average 45 days in duration (see page 602) and the egg to adult mortality to be uniformly 90 percent (see page 622). In 1939 squash plants were carefully examined at weekly intervals and all the bugs present were counted and characteristically marked with yellow enamel so as to determine the time of residence in the field before departure for hibernation and the effect of this upon the degree of parasitism. Obviously, the longer a bug remains in the field, the greater is its chance of becoming parasitized. It was found that of the number of bugs maturing within a given week, an average of 38 percent

remained in the field the second week, and 8 percent remained the third week. This information is utilized in the calculation of the above data which yields the following estimates of percentage of bugs effectively parasitized by the second and third generations of *T. pennipes:*

1937	25%
1938	32
1939	24

From the point of view of biological populations, there may be a wide difference between the apparent effect and the real effect of an entomophagous parasite. To maintain a steady state, it is assumed that for each pair of insects of one generation (assuming a sex ratio of .5), one pair of insects of the next generation survives to reproduce its kind. Consequently, in such a balanced state for *A. tristis*, there must occur a 99.2 percent mortality (assuming a reproductive potential per female of 241) which can be attributed to all the destructive factors impinging upon all stages in the development of the insect. The real destruction shared by each factor is usually much less than the apparent. This has been properly emphasized by Thompson (1928), who has postulated that if the factors contributing to this (99.2 percent) mortality act in sequence, the total fraction of the population present at the beginning of the life cycle, destroyed by all the factors taken together, can be represented by the equation:

$$D = (.992) = a + (1-a)b + (1-a)(1-b)c + (1-a)(1-b)(1-c)d + \ldots \text{ to x terms,}$$

when there are x factors involved.

Although the apparent magnitude of the factors acting destructively upon the squash bug are not precisely known, an estimate of the real magnitude, based on the above formula, may be suggested:

	Apparent mortality			Real mortality		
	1937	1938	1939	1937	1938	1939
Suppression of oviposition by *T. pennipes*	28%	3%	11%	28.0%	3.0%	11.0%
Egg-nymphal mortality	90	91	87	64.3	88.3	77.4
Adult parasitism	25	32	24	1.9	2.8	2.8
Winter mortality	?	?	?	5.0	5.1	8.0
				99.2%	99.2%	99.2%

On the basis of these figures, the total effect on mortality attributed to *T. pennipes* is 29.9 percent for 1937, 5.8 percent for 1938, and 13.8 percent for 1939, for which the first generation appears most responsible.

One other fact of importance, however, alters this conclusion. The above formula assumes that each factor is a discrete entity acting in sequence independently of the other factors. In regard to the suppression of oviposition of the squash bug, this obviously is not the case, for had not the first generation of parasites inhibited the oviposition of the host

to the extent noted, the additional eggs which would have been deposited would have been subject to the egg-nymphal mortality of 90 percent, 91 percent and 87 percent for 1937, 1938, and 1939, respectively. Accordingly, the reduction in offspring of *A. tristis* by the first generation of the parasite —*exclusive of natural mortality*—amounted to only 2.8 percent for 1937, .3 percent for 1938, and 1.4 percent for 1939.

A similar decrease in the mortality effects of the second and third generations of flies is caused by the factor of winter mortality, but the fraction of the original population so affected is relatively small as compared with the factor of the egg-nymphal mortality. It has already been noted that winter mortality does not change the relative numbers of parasitized and non-parasitized bugs, but winter mortality may cause the death of many bugs which might erroneously be attributed to the parasite.

From another point of view, that regarding the feeding population, the value of the first generation of parasites more nearly approaches the 28 percent, 3 percent and 11 percent levels, for the natural mortality is not of immediate importance. Since nymphs of all stages constitute the major portion of the feeding population, any factor tending to reduce the number of eggs hatching into nymphs does reduce the damage caused to the food plants. To be sure, the above levels are decreased by the mortality of the egg stage and by the cumulative mortality factors acting throughout nymphal development; but in reducing the feeding population the value of the parasite is significant.

The unusual abundance of *T. pennipes*, causing at times an apparent parasitism of as much as 85 percent or more, thus appears misleading when the real value of its effect is considered. But in spite of the apparently low order of magnitude of the real value, the parasite may have a profound effect on the steady density of the squash bug population. Throughout the writings of Nicholson (1933, 1937), Nicholson and Bailey (1935), Thompson (1928), and Volterra (1928), it is made obvious that mortality factors caused by meteorological conditions or weaknesses intrinsic within the organism itself act independently of the numerical abundance of the insect species. On the other hand, competition and the destructive capacity of parasites and predators are to a large extent dependent upon the numerical abundance of the species.

In the relationship under discussion, the power of increase of the parasite is so much greater than that of the host that it cannot be considered a limiting factor of parasitism. Moreover, the searching ability of the parasite, being of a random nature (at least towards adult bugs), is more efficient against a large host population, for fewer eggs are wasted through superparasitism. Accordingly, the destructive capacity of *T. pennipes* should vary directly with the population density of *A. tristis*.

It has been shown that while predation against the squash bug may occur, this factor is probably an insignificant one in controlling the insect.

Intraspecific competition for food and space appears to have little significance for the squash bug in this locality. Rarely are squash plantings entirely destroyed by the efforts of the bug alone, and while interspecific

competition for food is more likely, even this is commonly not of importance.*

Consequently, the parasitic factor gives evidence of being the only factor which regularly acts more severely when the population density of the squash bug is high. And since in the maintenance of the steady density, the action of the controlling factor must be governed by the density of the population controlled (Nicholson, 1933), *Trichopoda pennipes* appears to be the factor most responsible for the level of the steady density of *Anasa tristis*. What the level in this locality would be without the action of the parasite, is, of course, not known. But it is highly probable that were it not for the parasite, the common squash bug would be a far more serious agricultural pest than it is at present.

SUMMARY

The common squash bug, *Anasa tristis* DeG. (Hemiptera, Coreidae), is a well-known garden pest widely distributed in the western hemisphere. The description of this insect, its synonomy, morphology, and life history are discussed.

A. tristis feeds on cucurbitaceous plants, causing localized injury to the leaves and stems. Cumulative effects may destroy a squash plant, particularly if the bug injury is associated with injury caused by other insects.

Although mating of the squash bug occurs in the spring and throughout the season, multiple copulation is shown to be unnecessary for complete fertility.

Oviposition is favored by higher temperatures. The seasonal oviposition, which reaches a peak in early July, reflects the population density of the squash bug. Considering averages, the oviposition of an individual bug is found to be a rectilinear function of its longevity, the full reproductive potential probably never being realized.

The mortality of nymphs and the winter mortality of the hibernating adult bugs are high.

The most important natural enemy of the squash bug is a parasite, *Trichopoda pennipes* Fabr. (Diptera, Tachinidae).

The description of this insect, its synonomy, morphology, and life history are discussed.

The eggs of the parasite are deposited on the surface of the host, principally on the abdomen. The larva, upon hatching, bores through the body wall to spend its larval life, attached to a tracheal branch from the metathoracic spiracle, within the body cavity of the host. The larva, when mature, emerges from the posterior end of the bug and pupates in the soil. The host usually dies within 24 hours after the emergence of its parasite.

* At least for the localities and times observed. There is no doubt, however, that at times heavy infestations of *A. tristis* and/or other squash insects occur to cause complete destruction of squash plantings before all bugs have reached maturity. At such times the parasitic factor and the competition factor, superimposed upon each other, combine to form the controlling factor mentioned in the succeeding paragraph.

Superparasitism is common, but the supernumerary maggots are destroyed, leaving one to mature in each host. Cannibalism is probably responsible for this elimination.

Older squash bug nymphs may become parasitized, but in such cases the maggot remains in the first instar until the host matures. The possible mechanism causing this suspended development is discussed.

Within the host, the larva causes no loss of function vital to the individual; death to the host comes only as a result of larval emergence.

Parasitic castration of the bug, the details of which are described, has no functional significance in the male, but does cause a reduction of oviposition by the female.

Although there is one generation of the squash bug each year and three generations of its parasite, the life histories of the two insects do not synchronize sufficiently for the parasite to maintain the squash bug at a low population density. Also, host finding by the parasite is not efficient, and much of the oviposition of the fly is wasted through superparasitism.

The value of *T. pennipes* in reducing the numerical abundance of *A. tristis* is discussed and estimates for the 1937, 1938 and 1939 seasons are offered.

APPENDIX

EXPERIMENTAL METHODS

Parasitization For many experiments it was necessary to know the precise time at which the parasite egg was deposited upon the host. For such controlled parasitism, nonparasitized squash bugs were introduced into cages containing fertile female flies. Although the oviposition of confined flies is somewhat spasmodic, the time of parasitization can be determined by close observation. The hours of the late morning or early afternoon are most favorable for oviposition by the parasite.

Surgical Technique A surgical technique has been used successfully on the squash bug in a number of experiments. All stages of the parasite larva have been successfully removed — even a third instar almost ready to emerge, as judged by the extent of melanization of the posterior stigmata.

The first successful operation on a bug for the removal of a parasite larva resulted from the crude method of pulling off a metathoracic leg and by means of a very small hook, "fishing" out the maggot through the coxal opening. Quite obviously such a method has numerous limitations. The observation that a bug can withstand considerable injury to the abdomen led directly to the following method of operation, used under a binocular dissecting microscope. A bug is etherized until all spontaneous movements cease. The surface of the body is swabbed with alcohol, which is allowed to dry. Two incisions are then made by means of a fine scalpel. One of these is in a ventral direction in the intersegmental membrane between abdominal segments III and IV, extending from a point just ventral to the level of the spiracular openings to an indefinite point approxi-

mately halfway down the pleural region. The other incision is at right angles to the first, and joining it with the intersegmental membrane between abdominal segment II and the metathorax. (Since abdominal segment I is represented merely by a tergite which is fused to segment II, the segments here concerned appear to be I and II, although in reality they are II and III.) By means of forceps the membrane between the

FIGURE 18. Diagram of ventral view of squash bug, showing incision made to expose parasite larva and gonad (shown attached by tracheal vessels to second abdominal spiracle).

thorax and abdomen is torn (rather than cut) in a dorsal direction, so that a rectangular flap, hinged at the spiracular level, is formed. If done carefully, no injury to the tracheal vessels results. The cavity exposed by the opening of the flap contains a gonad, which is clearly visible. If a maggot is present on the side of the operation, it too, is visible or can be brought into view by slight manipulation of forceps or probe. The

position of the incision, maggot, and gonad are shown diagrammatically in Figure 18. Due to the fact that the maggot has an equal chance of being on one side or the other, operations concerning the maggot theoretically have a 50 percent chance of being successful. The author always opened the left side of the bug because of the convenience of operating and the better illumination when the thorax of the bug (ventral side uppermost) was held by forceps in the left hand. When the operation is completed, the flap is returned to its original position and held in place until the wound is sealed by the coagulation of the haemolymph. Although the bug exhibits movements within a relatively short time after the onset of anaesthesia, the effect of the ether is slow to wear off, and normal activity of the bug is not resumed for several hours.

Transplantation Experiments

Not only can parasite larvae be removed from the host without killing the latter, but a larva from one host can be introduced into another by the same technique. Also, a first instar maggot can be introduced into a new host by an injection method. The maggot is dissected from one host and placed in insect Ringer's solution. A glass tube, drawn out to a capillary having a bore slightly larger than the diameter of the maggot, is sharpened by bevelling at the capillary end and connected to rubber tubing and a mouth-piece at the other. Surrounded by Ringer's solution, the maggot is sucked up into the capillary, which is then inserted through the body wall of the new host. A slight pressure exerted by blowing through the rubber tubing forces the maggot into the body cavity. By coagulation, the haemolymph of the bug quickly seals the small opening made by the capillary.

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PLATE 1. A. Egg mass of squash bug. About 1.5 times natural size.*

B. Different stages of squash bug on leaf of summer squash. Slightly reduced.*

C. Dorsal view of adult squash bug. Twice natural size.

D. Dorsal views of ♀ (above) and ♂ (below) *Trichopoda pennipes*. About 1.5 times natural size.

E. Lateral view of squash bug bearing eggs of *Trichopoda pennipes*. Twice natural size.

F. Adult squash bug and newly emerged parasite larva, showing comparison of size. About 1.5 times natural size.*

*Photograph by B. H. Walden.

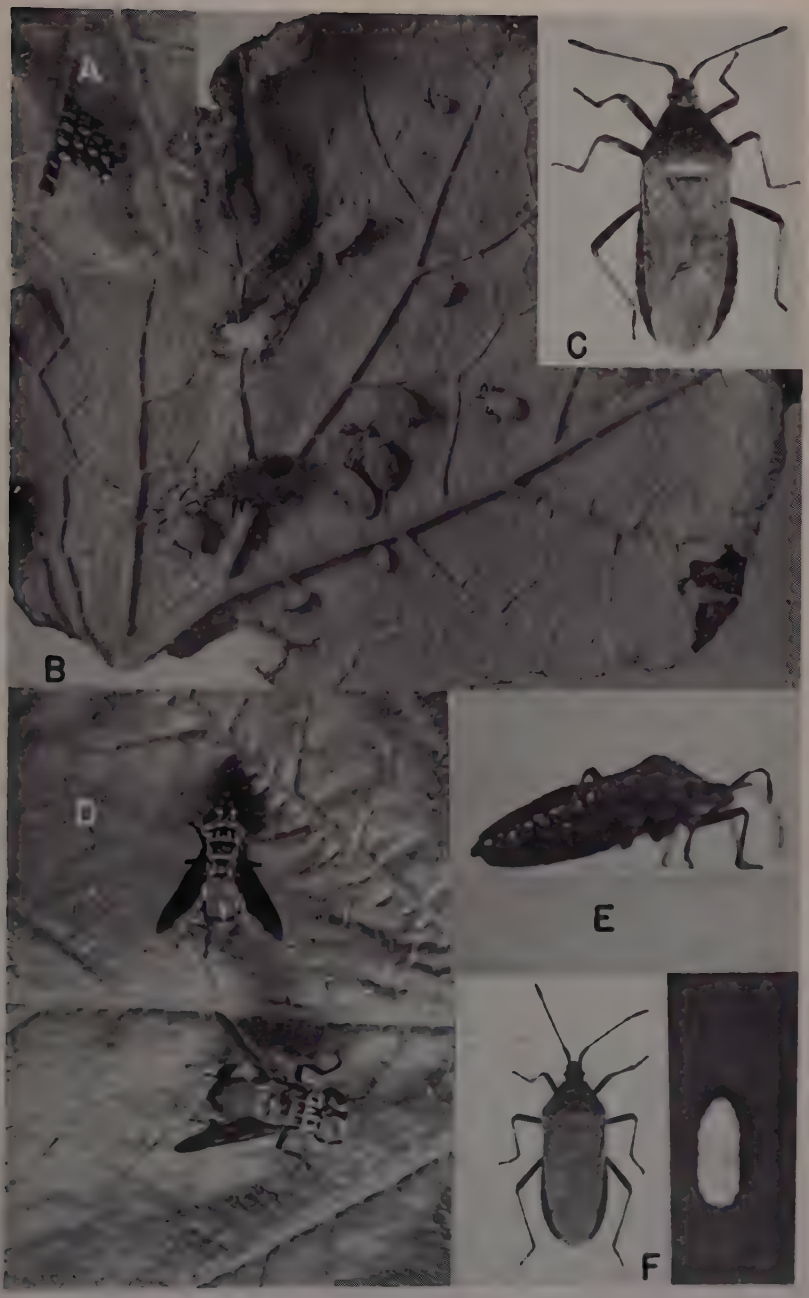
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PLATE 2. A. Cross section of squash stem, showing setal path of squash bug. X 160.

B. Cross section of squash leaf, showing feeding injury by squash bug. X 270.

C. View of leaf of summer squash, showing "burning" effect caused by extensive bug feeding. Somewhat reduced.*

*Photograph by B. H. Walden

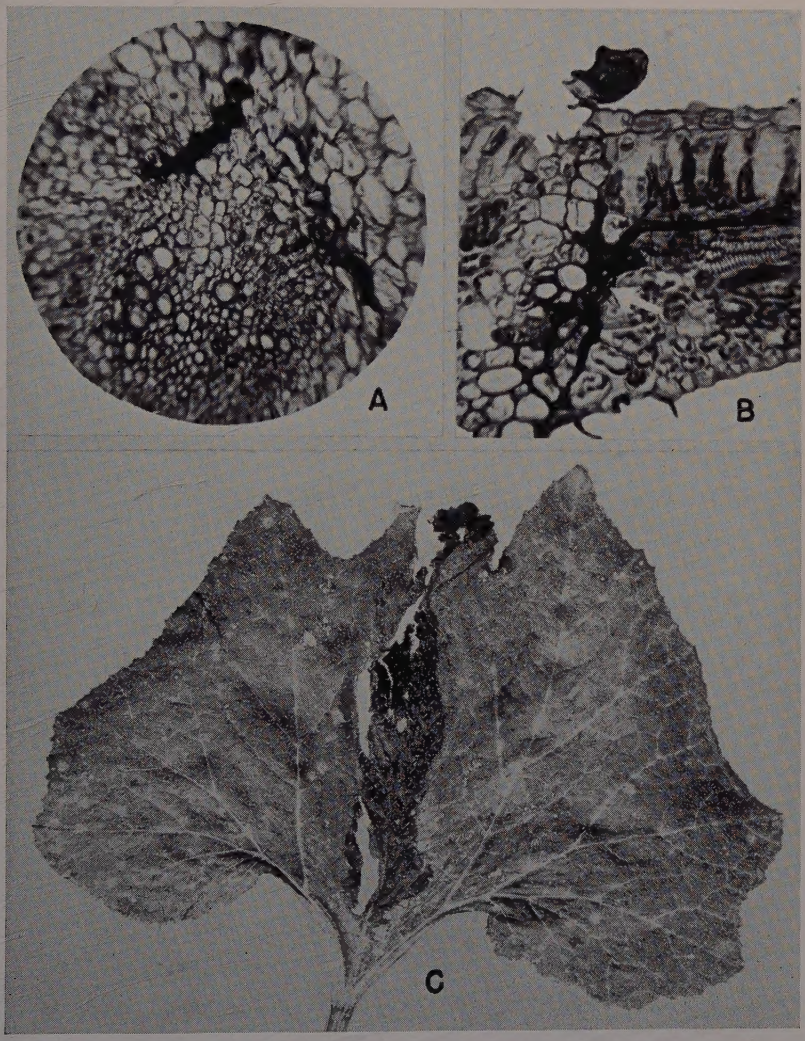
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PLATE 3. A. View of normal ovary of squash bug. X 12.

B. View of atrophied ovary from parasitized squash bug. X 12.

C. Longitudinal section of normal ovariole. X 24.

D. Longitudinal section of ovariole from parasitized bug, showing initial injury. X 20.

E. Longitudinal section of ovariole from parasitized bug, showing advanced injury. X 18.

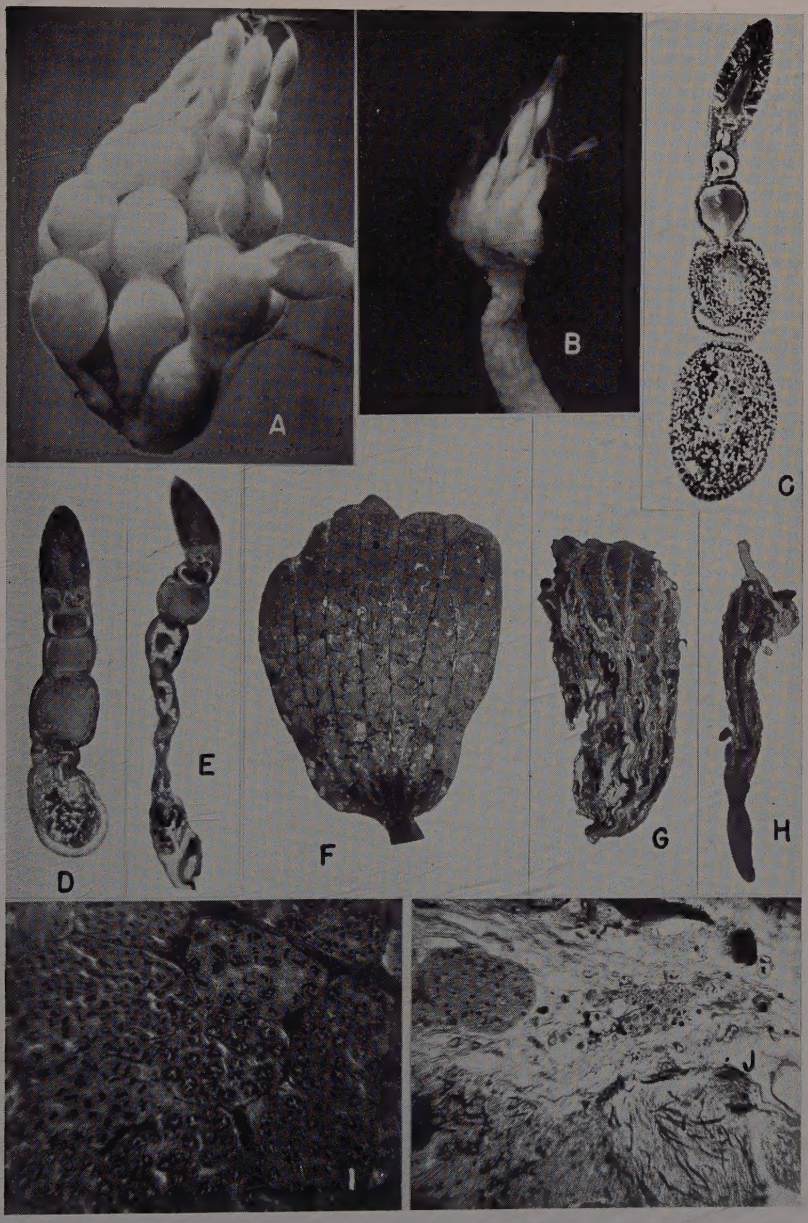
F. Longitudinal section of normal testis of squash bug. X 30.

G. Longitudinal section of partially atrophied testis from parasitized bug. X 27.

H. Longitudinal section of completely atrophied testis from parasitized bug. X 26.

I. Section of normal testis, enlarged. X 210.

J. Section of atrophied testis, enlarged. X 210.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